Aflatoxin: Impact on Stunting in Children and Interventions to Reduce Exposure

Co-organized by
International Food Policy Research Institute
and the
Enteric and Diarrheal Diseases, Agriculture and Nutrition teams
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Executive Summary

The International Food Policy Research Institute (IFPRI) in partnership with the Enteric and Diarrheal Diseases (EDD), Agriculture and Nutrition teams at the Bill & Melinda Gates Foundation (BMGF) brought together a group of multidisciplinary experts to explore the relationship between mycotoxin exposure and growth retardation in infants and to identify potential areas for collaboration and future research efforts. An additional goal was to develop links and exploit synergy and partnerships among those interested in agriculture, health, and nutrition, and policies and interventions to address this problem. To this end, there was an opportunity for the meeting participants to stay after the presentations and discussions on February 2 to interact with one another and develop collaborative efforts that could be submitted in the form of letters of interest (LOI) to the BMGF.

This report summarizes the two plenary sessions of the meeting (Association between Aflatoxin and Stunting, and Setting the Research Agenda) and the two concurrent sessions sponsored by the BMGF that focused on understanding the impact of mycotoxins on gut function and stunting (Potential Impact and Models, and Mechanisms and Biomarkers). This report does not summarize the two concurrent sessions hosted by the IFPRI on interventions to reduce aflatoxin exposure. This report is also intended to be used as part of the process of developing a white paper.

The meeting participants emphasized that the burden of stunting is enormous with more than 10% of total global burden of disease being attributable to maternal and child under nutrition. Annually, under-nutrition is responsible for the deaths of 3.6 million children under 5 years of age and for 140.5 million DALYs of children in that age group as well. The burden of mycotoxin exposure is particularly high in the those parts of the developing world where the population’s diet is limited to mainly a single food stable that is vulnerable to fungal infection, either in the field or post harvest during processing or storage. Consumption of aflatoxin contaminated food is associated with stunting in both stature and in cognitive development and likely associated with susceptibility to disease and reduced response to vaccinations. The key period of vulnerability to mycotoxins is not clearly defined, but includes both the pre-natal and the first few years of life; however the negative effects have life-long implications for the affected children. Different types of mycotoxins may have different mechanisms of action, and lifelong exposure is common in many areas. Only about 35% of stunting can be directly attributable to studied health and dietary factors, and the role of mycotoxins in the remaining 65% is not currently known. Stunting is likely to reflect a multifactorial process (nutrition, environment, genetics, infections, maternal factors) and thus teasing out the aspects related to any specific factor is difficult. The most studied mycotoxins are aflatoxins (from Aspergillus species), and fumonisins and deoxynivalenol (from Fusarium species). However, there are likely many more exposures to other mycotoxins in food.

Inflammation may be a common mechanism by which mycotoxins cause pathology that affects the health of people, especially children. Although at a molecular level, the pathway for these changes may be different for each mycotoxin, there may be commonalities in the systemic effects that need exploration. Another
common mechanism may be effects on intestinal integrity which can have deleterious effects on the adsorption of nutrients, allow for increased permeability to infectious agents as well as adversely affect the gut immune system.

Among the environmental factors where there has been a clear interaction with mycotoxins is hepatitis B virus where exposure to both factors results in increased risk for hepatocellular carcinoma. The sensitivity of individual persons and populations to the effects of mycotoxin exposure is likely affected by genetic factors.

Interventions face a number of barriers including the need to be tailored to the specific agroecological, economic, regulatory, educational, and social milieu of the country of deployment as well as to the ethical issue that testing and use preventative strategies have a higher level of safety requirements than do therapeutic strategies. Some simple promising chemopreventative strategies are currently under study.

Areas in which further knowledge is required include a demonstration of the causality of mycotoxin exposure and stunting. Hypotheses have been put forth for the molecular mechanisms and physiological mechanisms by which exposure to the different mycotoxins can lead to stunting. The proposed mechanisms involve systemic, multi-system, multi-organ as well as organ-specific effects. Effects on gastrointestinal integrity and the immune system are particularly important and can lead to under-nutrition, susceptibility to disease, poor response to vaccines and inflammatory processes. However, further work is needed to prove and delineate these mechanisms so that they can be taken into account in designing epidemiological studies and intervention strategies. Biomarkers and assays for them are available for aflatoxin, but different assays measure single or multiple molecules and use different instrumentation, which makes cross comparisons between studies difficult. Biomarkers for the fumonisins and DON, respectively, have only been recently developed and quantitative relationships between intake and the biomarker for both toxins have now been demonstrated.

Determining mycotoxin exposure is a complex: there is variability of exposure to mycotoxins by study site as well as within the site; there are seasonal effects on levels of exposure; there may be effects from both acute toxic exposures as well as cumulative effects, especially for aflatoxin which is fat soluble. The dosage that leads to biological effects in humans may differ because of the multifactorial nature of some of the effects. Although the adverse effects of mycotoxin exposure are most severe in the developing world, exposure to and deleterious effects of mycotoxins are also seen in the developed world. The regulatory processes and exposure limits also differ by country and regulatory body.

A number of different animal model systems have been used in these investigations. Some animals, such as the piglet, have more similarity to human physiology, but are limited in terms of the number of animals that can be studied. Rodent models, including KO mice, can provide useful information, but their metabolic and immune systems have important differences from those of humans.

Among the possible interventions to reduce aflatoxin exposure are pre-harvest agricultural practices, post-harvest sorting and storage practices, use of hepatitis B vaccine, and dietary changes.

Studies to assess the burden, mechanisms, and intervention strategies, are labor intensive and require an in-country presence and infrastructure that requires time to develop. Given this, the potential for using extant samples from prior studies or to build add-on to ongoing projects, such as MAL-ED, will be important for efficiently obtaining the needed data.
Summary of Sessions

**February 1, 2012**

**Introduction**

The meeting participants were welcomed by Dr. Maximo Torero, Division Director, Markets, Trade and Institution Division of the International Food Policy Research Institute and by Dr. Gretchen Meller, Program Officer, Enteric and Diarrheal Diseases, Global Health Program, Bill & Melinda Gates Foundation.

The International Food Policy Research Institute (IFPRI) in partnership with the Enteric and Diarrheal Diseases (EDD), Agriculture and Nutrition teams at the Bill & Melinda Gates Foundation (BMGF) organized this meeting to bring together a group of multidisciplinary experts to explore the relationship between mycotoxin exposure and growth retardation in infants and to identify potential areas for collaboration and future research efforts. The objective is to better understand and define the health risk contributed by mycotoxins (such as aflatoxin, fumonisin, and deoxynivalenol (DON)) in children under five and in utero, specifically related to the integrity of gut function that results in stunting with the goal of developing a better understanding of the impact of mycotoxins on gut function and stunting. Additionally, the goal is to develop links and exploit synergy and partnerships among those interested in agriculture, health, and nutrition, and policies and interventions to address this problem. There is potential for development of collaborative and companion projects with the Malnutrition and Enteric Diseases (MAL-ED) project funded by the BMGF to examine the hypothesis that infections early in life lead to malnutrition by causing inflammation and/or by altering intestinal barrier and absorptive function and that this leads to failure of normal growth and development.

A planned outcome of the meeting was the production of this summary, which can be used as part of the preparation of a white paper; it will take a number of additional months to develop the white paper. Moreover, there will be an opportunity for the meeting participants to stay after the presentations and discussions on February 2 to interact with one another and develop collaborative efforts that could be submitted in the form of letters of interest (LOI) to the BMGF, which is currently looking at developing new strategies to move forward in this area.

This report summarizes the two plenary sessions of the meeting (Association between Aflatoxin and Stunting, and Setting the Research Agenda) and the two concurrent sessions sponsored by the BMGF that focused on understanding the impact of mycotoxins on gut function and stunting (Potential Impact and Models, and Mechanisms and Biomarkers). This report does not summarize the two concurrent sessions hosted by the IFPRI on interventions to reduce aflatoxin exposure.

**Plenary Session 1: Association between aflatoxin and stunting**

**Overview of stunting: determinants and unknown contributors**

*Marie Ruel*

Dr. Ruel stated that stunting is defined as low height-for-age (< -2 SD). It is a cumulative process that results from prolonged exposures to poor health, food, and care and is largely irreversible after 2 years of age.

Worldwide, 171 million children under 5 years of age are stunted, with the largest problem being in sub-Saharan Africa and South Asia. In the past 20 years (1990-2010), progress has been made globally in reducing the prevalence of children under 5 years of age affected by stunting from 44% to 29%. The major
reductions in stunting prevalence have been in Asia and Latin America, but no improvement was observed in Africa, where in fact, the number of children affected by stunting has actually increased over time – from 45 in 1990 to 60 million in 2010. In looking at global patterns of under-nutrition, children from developing countries are born shorter than average (compared to WHO standards), and their height-for-age Z-scores (HAZ) continue to decline rapidly in their first two years of life, reaching a low level of approximately -2 Z-scores by 2 years of age and remaining at this level afterwards. The lowest HAZ are found in Sub-Saharan Africa and South Asia. The slow growth in height-for-age is seen as early as during pregnancy (as seen by low HAZ at birth), thus, the well-recognized need to focus on the first 1000 days, pregnancy up to the child’s second birthday in order to effectively prevent stunting and the irreversible damage it does to the lives of those affected.

There are a number of reasons why preventing stunting is important, including issues of welfare, human rights and equity, and survival. The burden of stunting is enormous with more than 10% of the total global burden of disease being attributable to maternal and child under-nutrition. Annually, under-nutrition is responsible for the deaths of 3.6 million children under 5 years of age (35% of deaths) and for 140.5 million DALYs of children in that age group as well (35%). From an economic perspective, there is a need to break the vicious cycle of under-nutrition and poverty as estimates suggest that failing to address them results in of 2-3% losses in national GDP; reduced physical productivity of the work force (due to short stature); reduced cognitive development; delays in starting school (7 months); losses of schooling (~0.7 grades); reductions in lifetime earnings; overall reduced economic productivity, wages and income; and in women, poor reproductive performance including smaller babies. Collectively, these factors lead to the inter-generational transmission of under-nutrition and poverty.

It is important to recognize that a child stunted at 3 years of age remains stunted at adulthood, even if the child starts to receive good nutrition at any time after 3 years of age. Perversely, over-feeding undernourished children after the age of 2-3 years, increases the risk of overweight and obesity, but does not result in catch up growth in height. By contrast, effective nutrition interventions in children before the age of three years have been shown to have long-lasting positive impacts on a series of outcomes. A study in Guatemala, for example, showed that improving early childhood nutrition had large and significant impacts on cognitive development during childhood and later in life, on the level of education achieved, and on economic productivity (~46% higher wages among those who were exposed to the intervention during their first 2-3 years of life). Importantly, the younger the children were at the time they were first exposed to the intervention, and the longer they were exposed to it during their first 2-3 years of life, the greater the effects were on the long-term outcomes. The underlying mechanism for this improvement appeared to be through improved cognition, schooling and attainment of skilled jobs rather than through improvements in height as previously believed. Dr. Ruel stated that this study is unique in that it included a rigorous randomized design for the nutrition intervention tested in the late 60’s and early 70’s, and achieved to find a large proportion of study subjects when they were young adults.

Dr. Ruel summarized several interventions that are known to reduce stunting. During pregnancy, these involve balanced energy-protein supplementation, multiple micronutrient supplementation and intermittent preventive treatment of malaria. During the first 24 months of the child’s life, improved complementary feeding, zinc supplementation and improved hygiene have been shown to be effective. The impact of universal coverage with all interventions is a reduction of 36% in the prevalence of stunting at 36 months of age. It is not clear what factors contribute to the remaining 64%, and how much exposure to mycotoxins may contribute to this.

**Estimates of global exposure**

*Felicia Wu*

Dr. Wu’s presentation focused on aflatoxin, a mycotoxin which is produced by *Aspergillus flavus* and *A. parasiticus* and contaminates such dietary staples as maize, peanuts, tree nuts, cottonseed, spices, and
The exposure to aflatoxin is highest in warm regions (Africa, Asia) where maize and peanuts are dietary staples. The human health effects associated with exposure to aflatoxin include liver cancer (it synergizes with chronic hepatitis B virus (HBV) infection and results in a much higher cancer risk than either exposure alone), childhood stunting, acute aflatoxicosis and modulation of the immune system.

In order to have a way to prioritize diseases and conditions for policy-making decisions, the World Health Organization (WHO) has developed a system to measure the global burden of disease (GBD) which uses DALYs (disability adjusted life years) as a metric. DALYs are the total of the Years of Life Lost, across all who suffer disease worldwide (YLL) and the Years Lived with Disability (YLD), which is multiplied by a weight [0 to 1] depending upon the severity of the disease or condition. DALYs are stratified by sex and high/middle/low income country. Childhood underweight is a major contributor to DALYs in many low income countries.

The WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) has examined the disease burden caused by chemicals (mycotoxins, seafood toxins, mushroom poisons, pesticides, metals, cyanide, allergens) and enteric and parasitic agents (E.coli, Salmonella, Campylobacter, Listeria) and had commissioned Dr. Wu to estimate global burden of aflatoxin-related disease. If one looks at liver cancer alone, globally, there are 25,200-155,000 aflatoxin-induced liver cancer cases per year, which represents about 3.4-21% of all cases of hepatocellular carcinoma (HCC). Assuming 13 DALYs per liver cancer case, this is 328,000-2,000,000 DALYs per year.

The association between aflatoxin exposure and childhood stunting is less well-defined. Childhood stunting is defined as a child’s height-for-age being 2 standard deviations or more below the WHO growth reference (HAZ ≤ -2). Childhood stunting is associated with cognitive impairment and increased vulnerability to infectious disease. There are 195 million stunted children under age 5 worldwide, with the highest burden in Sub-Saharan Africa and South Asia.

There are a number of causes of childhood stunting including: genetics, nutritional factors (energy intake, macronutrients, micronutrients), infections (which result in injury to gastrointestinal mucosa / epithelium, systemic effects [repeated diarrheal diseases], immune modulation), maternal factors (maternal nutrition, behavioral factors) and quite likely dietary and environmental toxins.

Dr. Wu summarized several studies linking aflatoxin to growth impairment in children. (1) Aflatoxin measurements in stored flour in rural homes demonstrated that stunting, underweight, and wasting was associated with higher aflatoxin (AF) levels in flour. (2) Cross-sectional studies of aflatoxin-albumin (AF-alb) levels in maternal, cord, and children’s blood demonstrated that stunting and underweight were associated with higher AF-alb levels in these fluids. (3) Longitudinal studies of AF-alb levels in children’s blood demonstrated that reduced height gain in 8 months was associated with AF-alb levels. (4) The presence of AFM1 in mothers’ breast milk was associated with lower length at birth and with the presence of AFM1 in infants.2

Dr. Wu stated that it was not possible to calculate the global burden of aflatoxin-related stunting at this time for several reasons, including the fact that the mechanism remains unknown, more studies are needed in different populations, there is a need to control for all confounders in such studies, and that ideally, interventional studies would be conducted. She noted that even if the calculation could be made, WHO

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1 Alfatoxin-albumin (AF-alb) is one of several biomarkers of aflatoxin exposure that have been developed from metabolic products of aflatoxin. AFB1-N7-Gua is another biomarker that has been studied.
2 The naturally occurring aflatoxins are AFB1, AFB2, AFG1 and AFG2. AFM1 and AFM2, the hydroxylation products of AFB1 and AFB2, respectively, are found in milk and milk products.
assigns stunting a low disability weight: 0.000-0.002 (on scale of 0-1). Importantly, how the global burden of aflatoxin-induced disease is estimated affects how the cost-effectiveness of interventions is evaluated.

Among the possible interventions to reduce aflatoxin exposure are pre-harvest agricultural practices, post-harvest sorting and storage practices, use of hepatitis B vaccine, and dietary changes. WHO calculates effectiveness as a function of GDP/capita and number of DALYs saved. Feasibility is also an important characteristic of an intervention, that is, can the proposed intervention get to and be adopted where it is needed. One needs to consider the basic characteristics of the intervention and its usage as well as the impact of governmental regulations and capacity and the ability to deliver the intervention.

To deal with all of these factors, Dr. Wu has moved into using a network model. This type of a model can take into account the various in-country factors (disease prevalence), crop factors (aflatoxin levels in crops) as well as international inter-country factors (e.g. trading partners, governmental standards and regulations, environmental events such as fire or drought) that effect the access to and usage of dietary staples.

She cited a number of areas where network approaches might be deployed in the future including: (1) Economics, food security and global health, for example, which nations are main food exporters/importers, do trade clusters emerge, how do food prices or mycotoxin regulations affect specific nations, or clusters of nations, and if fire, drought, or plant disease occurs in a particular nation, which other nations are also affected. (2) Interventions, for example, if a crop is only produced in one part of world, determining if it can get to where it’s needed and defining where goods should be sourced in emergencies. (3) Biology, for example, determining how toxins and infectious agents synergize to cause stunting.

**Highlights from Aflacontrol Project: Aflatoxin prevalence levels and household knowledge, attitudes, and perceptions**

*Clare Narrod*

Dr. Narrod reported on a project to explore the scope of cost-effective aflatoxin risk-reduction strategies in maize and groundnut value chains to improve market access and health of the poor in Africa. The motivation for these studies is that although the economic losses are estimated to be large, there is a dearth of systematic studies that empirically estimate the economic losses (health, income) for all stakeholders along the value chain, the economic impact of interventions and the socio-economic factors affecting adoption of interventions. Although there have been a number of biological studies on control options; there hasn’t been large scale adoption of these interventions.

ACDI/VOCA\(^3\) has observed that in Kenya, poor producers are the least likely to adopt aflatoxin risk reduction technologies since they lack the necessary resources, and, thus, they are the group most susceptible to aflatoxin exposure. A multidisciplinary team was employed that assessed multiple factors including: economic impact, perception of aflatoxin and willingness-to-pay (WTP), and risk analysis.

Groundnut areas, Mali and Kenya, were studied. Prevalence data were collected from different agro-ecological zones (AEZs) from 2009-2011 (Kenya) and 2009-2010 (Mali) at pre-harvest and in storage (15 to 30 days intervals), and in the markets (every month). Livelihoods impact was studied though qualitative focus group surveys and quantitative household surveys (both household surveys and community level surveys), as well as by socio-economic data collection. Aflatoxin prevalence levels (ppb) were mapped and risk analysis was undertaken. Using the data that was collected (known locations of aflatoxin and environmental layers), a widely-used and accepted ecological niche modeling program, Maxent Model, as

\(^3\) ACDI/VOCA is a private, nonprofit organization that promotes broad-based economic growth, higher living standards and vibrant communities in low-income countries and emerging democracies.
used to identify a probability map of species potential distribution, and information on importance of environmental variables (sensitivity analysis). Statistical evaluation of the model was also undertaken.

Dr. Narrod stated that the Maxent model has several limitations, for example: (1) predicted prevalence was clustered reflecting the same range of prevalence levels in a certain location and (2) it is not clear if the model captured the full range of environmental conditions acquired; if the model did not capture the full range of environment conditions, it would not accurately predict the full range of the aflatoxin infection. (3) It is also not known whether factors other than the environment are influencing aflatoxin levels. In this regard, some data indicated that households next to each other had different levels of aflatoxin. Other factors, e.g., initial levels in soil or operational issues, such as, drying and storage practices) may also be influencing aflatoxin levels.

Dr. Narrod summarized the characteristics of the study population, of the production and storage practices, and of the sources of information about aflatoxin. She stated that the results of structural equation modeling showed that: (1) Household head’s level of education had a positive and significant effect on the perception of risk and spread. This suggests that educational campaigns are needed to reach those who don’t necessarily have access to formal education methods. Given that most of those who had heard of aflatoxin heard about it through local language radio, efforts to expand the effectiveness through this medium are needed. (2) Household head’s level of education had a negative and significant effect on the household’s reaction to the extension officer or public health office regarding a potential risk in the village. This finding may reflect that higher educated households take less stock in what extension officers say than less educated households and point to the need to improve the credibility of public service officers regardless of education level. (3) Having children less than 5 years old in the household has a positive effect on actual use of some sort of storage method and on knowledge of the attributes associated with safe consumption of food for human and animals. (4) Female household heads have higher knowledge about harmful effects of feeding moldy grains to animals and eating maize products. (5) The income effect is negative for knowledge of safety attributes, but positive and significant on knowledge of causes associated with moisture and perception of risk, a finding that was expected. (6) Households in the dry lands, which is where the 2004 outbreaks occurred, had higher perception of risk indices as expected, but negative indices on knowledge of safety attributes, knowledge of moisture causes, and actual actions in terms of storage uses. The negative effect may actually reflect a lack of understanding of the problem and of ways to prevent aflatoxin in that region. (7) The scale of operation, that is, the size of land cultivated or its value or production had no effect on farmers’ actions. (8) Being involved in selling maize had no effect in terms of actions to reduce risk.

Dr. Narrod described several areas where there are gaps in knowledge and where additional work might be needed. With respect to social networks, more information is needed on the effect of social networks on knowledge of aflatoxin, on which networks can be effective engines in disseminating knowledge and about how networks can be useful in turning knowledge and awareness to correct actions. It is also not clear whether increases in knowledge and awareness about aflatoxin reduce prevalence levels. Further work is needed on the impact of education/radio campaigns. Randomized controlled trials are needed to determine the effectiveness of education and awareness campaigns in changing behavior with respect to adopting risk mitigating practices.

Child Health and Consumption of Aflatoxin Contaminated Foods: A Review of Two Studies that address the link between aflatoxin and stunting
Kitty Cardwell

Dr. Cardwell reported on studies that were done in 2000-2001. The concept for these studies started in the 1990’s when she was working with the International Institute of Tropical Agriculture (IITA) and was concerned about aflatoxin that was present in corn in West Africa. There was no consumer awareness or regulatory functions related to this issue. Following a meeting in 1995 in Benin at which the levels of aflatoxin in corn were discussed, a white paper was developed on measuring the aflatoxin and its public
health effects. Individuals from both the agriculture and health sectors worked with each other, which is difficult to achieve.

Two studies were undertaken. The 2000 study (Study 1) was a cross-sectional study of infants in 16 villages in 4 ecological zones involving 30 children per village (480 children). Measurements included blood albumin-aflatoxin adducts, anthropometrics, and 200 covariate factors. The 2001 study (Study 2) was a cohort study of 200 children from the 2000 study and involved 100 children from the two highest exposure-risk villages and 100 children from the two lowest exposure-risk villages and three sampling periods (every 3 months). Measurements included blood albumin-aflatoxin adducts, anthropometrics, and over 200 covariate factors. In Study 1, the children were 9 to 48 months of age; in Study 2, they were 16 to 37 months. Parental informed consent was obtained as were approvals from the Ministries of Health in Togo and Benin. All children had birth registration cards, most (80%) were receiving vaccinations, and all children were dewormed at the study onset to avoid the effects of helminth infection. In Study 2, all children were given HBV vaccination.

The pg/μl blood albumin-aflatoxin in 6-48 month old infants in 16 villages in Benin and Togo varied by region. The mean in the Sudan Savanna (SS) was 25 pg/mug; in the Northern Guinea Savanna (NGS) was 55 pg/mug, in the Southern Guinea Savanna (SGS) was 129 pg/mug and in the Coastal Savanna (CS) was 43.65. The highest levels were 249 pg/mug in Lainta; the next was in Ye with 129 pg/mug. All of the children in all of the zones did have some level of aflatoxin in their blood.

Dr. Cardwell described the results in terms of the significance of demographic variables in regression against blood aflatoxin. The population involved 10 ethnic groups but differences in blood adduct levels became insignificant when village and zone were added. The religion of the household head determined the educational level of the mother in all zones. With higher mother’s education, there was significantly less *Aspergillus flavus* in household foods. In the SGS, animist households had significantly more blood aflatoxin than other religions; however, this variable was confounded with village. The average number of reproductive age women per household was 2.6 with an average age of 28.5. The woman’s position in the household was not a significant determinant of infant blood aflatoxin levels. The mother’s economic status was more highly significant than that of the household head in terms of infant blood aflatoxin levels. In the NGS, adduct levels increased with the number of siblings.

Dr. Cardwell summarized the effects of the MumScore, which is a composite index of the mother’s possession of productive and consumptive assets. Richer mothers are more likely to feed children maize throughout year and to have it in store. Mothers with higher scores (particularly in the NGS) had higher frequency of groundnuts available during the year and were more likely to have it in store. On average, children consumed maize more than 6 days/week and groundnuts more than 3 days/week regardless of wealth. Higher SES significantly negatively correlated with how much fungus was in the stored foods, thus, with lower adduct levels.

Dr. Cardwell summarized the significant variables in regression against anthropometrics across zones. Factors affecting the HAZ were maternal education, moms’ body mass index (BMI), child’s birth-weight, aflatoxin-albumin adduct, and absence of younger sibling. Factors affecting the WAZ were maternal education, moms’ BMI, child’s birth-weight, and albumin-aflatoxin adduct and factors affecting the WHZ were maternal education, moms’ BMI, child’s birth-weight, weaning status, and recent or current fever. Negative factors were the level of aflatoxin and blood parasite density (malaria).

Dr. Cardwell presented a summary of the food basket, nutrition and health status relative to anthropometric data. Twenty-nine foods were assessed per household. Only yams were associated with lower mycotoxin levels. It was not possible to do quantity analysis because more than 60% of the households used a common plate. No analysis was done of total food by anthropometrics by SES. Health data were recorded on all children during all visits, however recall data was disallowed. Only physical parameters; fever, parasitemia,
and blood hemoglobin counts were strictly relevant. It is in these factors where the most uncertainty lies as there is not information about the full nutritional diversity of the children and how much the individual child was eating.

Dr. Cardwell noted that while one might assume that a higher MumScore would lead to greater diversity in the food basket, more food, and better general nutrition, regression models did not show the MumScore as a significant factor relative to growth variables.

Dr. Cardwell noted the importance of the zone and village in pre-harvest and post-harvest management. The practices are dependent on the village, with everyone in village using the same methods; thus, an effective intervention would likely be adopted by the whole village.

Dr. Cardwell concluded by stating that humid forest and mid-altitudes are likely to be exposed to fumonisins, DON, and trichothecenes. Moist savannas (particularly with bimodal rainfall patterns) and hot dry savannas have high risk of significant levels of aflatoxin exposure where maize is a staple in the diet. Thus, a large part of sub-Saharan Africa is at risk for significant mycotoxin contamination. It is important to determine the aflatoxin strains in the study area as the S strain is always highly toxic, while L strains are often less toxic.

**Discussion**

The following points were made during the discussion of the first four presentations.

At the population level, once the trajectory to stunting occurs, there seems to be no recovery, either in humans or in animals. The first three years of life are the most important and interventions need to be undertaken as early as possible.

It was noted that the Meridian Institute has just completed a grant agreement with the BMGF to support the Africa-led, Partnership for Aflatoxin Control in Africa (PACA). The BMGF funding to Meridian includes support for a number of activities including country assessments, scaling up of beneficial fungi (Aflasafe), improvement and implementation of low-cost methods for post-harvest storage, drying and handling, and studies of economic impact and health.

In terms of the focus on aflatoxin and not other toxins in the BMGF projects, it was noted that aflatoxin has more severe health consequences. The original projects related to trade and market access, and health was later added-on. Also, it was felt that it was more strategic to focus on one mycotoxin when trying to go from agriculture to nutrition. However, it is recognized that a broader focus may now be relevant, but if efforts are diluted into too many toxins, it may not be possible to develop effective interventions.

It was also noted that it is important to distinguish between toxicology and exposure in considering the knowledge about the health problems associated with mycotoxins. Also, the absolute level of a toxin may not be as important as the dose effect. In some cases in Africa, it is the level of mycotoxin that it is killing children. However, it is important to recognize that the children are accumulating toxin as they get older and the effects seen may be the result of the cumulative exposure.

It is important to determine the strain of aflatoxin in the region under study. For example, in Kenya in regions that have maize high in aflatoxin, some regions have more S strains, which are highly toxic and other regions have more L strains, which may be less toxic. A lot of the work on aflatoxin has focused on cancer as an endpoint, because a lot of NIH funding was available in that area. A lot of the other biologic effects of aflatoxins remain to be explored and separated from confounders.
Fumonisins were discovered in 1989 and have a different mechanism of action than aflatoxin. Although the levels of fumonisins go down during food preparation, there is still a lot left after cooking. There are data emerging on the association of fumonisins and stunting. Although there has been work on biomarkers for aflatoxin for a long time, fumonisin biomarkers are just being developed. Such biomarkers will be available as tools. While there is not as much information about DON in humans, there is a fair amount of knowledge about DON and other toxins in animals. In terms of which mycotoxins might be studied, it was noted that in the poorest countries, aflatoxin is more common in food supplies. DON is more of a problem in cooler and more temperate climates. Fumonisins are in between aflatoxins and DON its prevalence. Dry Savanna and Sahel, and moist Savanna have aflatoxin, whereas forests and Savanna will have fumonisins. There are some areas where high levels of aflatoxin are present and which also have fumonisin present. As food is weathered, there are more toxins present.

Although the Codex does set guidelines for aflatoxin in food stuffs, each nation can sets its own standards and there may not be monitoring at the local level. Additionally, in some areas where there were high levels of local contamination with mycotoxins, the adjacent countries had the same problem and so it is not possible to have ready access to uncontaminated food. It was noted that in Guatemala, the trade within the country is important; not only does the corn move between the highlands and lowlands, but the people also move between these two geographic zones, making it difficult understand the exposure levels of a particular person and also to develop interventions. Thus, in terms of mycotoxins, the problem is not food stuffs traded internationally, but rather the local production that isn’t traded internationally, and is consumed locally. For example, of the total peanut production around the world, only 5% is exported, and 95% is used locally. Even when maize comes from the U.S., there can be delay at the entry points and the maize may be stored under poor conditions and so may become contaminated with aflatoxin.

The amount of mycotoxin contamination of maize and ground nuts depends on the season and situation, with rainfall and moisture driving higher levels of contamination. In some areas, as much as 90% of product is not acceptable. In studies of small farmer access to markets, it was found that 50% of purchases from small farmers were rejected because of aflatoxin. Often the regulations on levels of contamination are not enforced. It was stated that it is important to have complementing work done on market access.

The exposure to aflatoxins may increase over time. In the map of Africa developed by IITA, maize is moving north from the moist Savanna and going to the dry Savanna and Saleh; it is in the latter area where the problem is becoming more acute. Fumonisins are prevalent in certain parts of Africa, e.g., there are high levels in Zimbabwe, in southern. It was noted that fumonisin exposure is regulated at ppm, and aflatoxins are regulated at ppb. In Tanzania, both aflatoxin and fumonisins are found in children and so there is the potential for synergic effects. In Guatemala, aflatoxin and fumonisins also co-occur.

Understanding the connection between malnutrition and enteric disease: Overview of the MAL-ED project objectives and early data  

Dennis Lang

Dr. Lang stated that malnutrition contributes to childhood deaths associated with all infectious causes. There is increased mortality from infectious diseases, including diarrheal diseases, in children who are underweight, stunted and wasted.

Environmental (or tropical) enteropathy is a condition that develops in individuals living in areas with limited access to clean water and sanitation. It has been characterized by changes in the small bowel including bacterial overgrowth, inflammation, villus blunting, decreased surface area, nutrient absorption, and increased permeability which increases access of bacteria or bacterial products to the circulation. This intestinal pathology appears to be reversible when individuals move to more sanitary conditions. Unfortunately, adequate definition of this pathology, which requires intestinal biopsy to fully characterize, is lacking in newborns and very young children most at risk.
The development of environmental enteropathy is thought to result from the almost continual exposure to pathogenic bacteria, parasites and viruses found in the environment, food and water. Dr. Lang described a “vicious cycle” of infection, gut damage, decreased nutrient absorption, malnutrition and decreased immune response which, in turn, results in increased susceptibility to repeated infection. A result of this cycle is often significant growth and cognitive deficits in the individual, which have both long term personal and societal effects. Other factors that likely contribute to this cycle and which may define individual differences in response to this environment are genetic polymorphisms, differences in the composition of the gut microbiome, and epigenetic effects on gene expression. Aflatoxin exposure could be viewed as just another factor in this challenging environment that contributes to this pathology. This cycle of repeated enteric infection (with or without resulting diarrheal disease) and malnutrition could be interrupted by interventions such as: antimicrobials, repair micronutrients, probiotics, prebiotics, access to clean water and sanitation and vaccines.

The MAL-ED study is currently underway in eight field sites in Peru, Brazil, Tanzania, South Africa, India, Pakistan, Bangladesh and Nepal. At each site at least 200 newborns are enrolled and intensively followed for two years. The hypotheses underlying the MAL-ED project are: (1) that infection (and/or co-infection) with certain enteropathogens leads to malnutrition by causing intestinal inflammation and/or by altering the barrier and absorptive functions of the gut; (2) that the combination of enteric infections and malnutrition results in growth and cognitive impairments in children and may lead to impaired immunity as measured by vaccine response; and (3) that particularly sensitive periods exist during early childhood when environmental exposure, infection, and malnutrition lead to exacerbated and lasting effects on development, both in terms of growth and cognition.

The research questions, measurements and outcomes are designed to define the contributions of infection, nutrition, and the social and physical environment on both physical and cognitive development in individual children. The outcome measures are of growth, vaccine response and cognitive development. In addition to the longitudinal cohort studies, two of the sites (Brazil and Bangladesh) are also conducting case-control studies, where cases are defined as underweight children (Z score of >-2) between 6 and 24 months of age. Enrollment is now complete and children are under surveillance for at least 24 months. All of the data discussed should be considered preliminary and interim. There may be opportunities for aflatoxin work to take place in this network.

The microbiology assessment protocol includes screening for 57 pathogens in all diarrhea and normal stool samples (bacteria, viruses, and parasites). Studies of the microbiome, in terms of development and changes in normal gut flora, are being done by Jeff Gordon’s group at Washington University and collaborating institutions as part of a companion project. MAL-ED study samples will be archived for future studies and for discovery projects.

Measurements of gut function include stool neopterin and myeloperoxidase (indicators of inflammation); dual sugar uptake and excretion in urine as a measure of gut absorptive capacity and permeability; stool alpha-1-antitrypsin as a measure of intestinal permeability, and possibly plasma EndoCAb antibody (IgG) as a measure of bacterial LPS translocation from the gut lumen to the circulation.

Dr. Lang presented an overview of the kinds of data that are being obtained in terms of disease burden at MAL-ED sites. He noted that in addition to diarrhea, information is being obtained through questionnaires administered by field workers about additional clinical signs. Pathogens are being assessed in both diarrheal stools and normal stools. Dr. Lang noted that a number of infectious agents, sometimes multiple pathogens simultaneously, can be detected in stools in the absence of any diarrhea. An individual child can also be

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4 The EndoCab ® ELISA was developed for determination of endotoxin core antibodies in human plasma or serum.
followed over time in terms of symptomatology and infection, and in that way serves as its own control. Dr. Lang stated that given these observations, additional thought may be required regarding the effect that these multiple pathogens are having on the physiology of the gut and consequences on growth and development.

The children are followed by twice weekly surveillance and questioning of care giver; this process allows for the collection of data relating growth as stunting or wasting of the child to episodes of diarrhea, bloody diarrhea, acute lower respiratory tract infections (ALRI), fever, and other clinical observations. Dr. Lang presented some examples of data that has been collected at the MAL-ED sites relating to stunting. In most of the study sites, the children are below average at birth and stunting increases over time till about 40-50% of children are stunted (> -2 HAZ) by two years of age at most sites.

Dr. Lang provided some examples of data relating to vaccination. The MAL-ED sites are maintaining records of immunizations and are measuring the immune response to EPI (Expanded Programme on Immunization) vaccines as an assessment of under-response to vaccines (e.g., children in India and Pakistan are getting polio vaccines but cases of polio are still reported in some areas.)

Dr. Lang provided some examples of data related to nutrition. The MAL-ED protocol looks to characterize the timing and nature of breast feeding practices, the introduction of weaning foods, and the quantitative estimation of nutrient intakes through the use of 24 hour food recall methodology. He stated that early data confirm that exclusive breast feeding is protective against diarrheal disease.

Dr. Lang stated that the primary goal of the MAL-ED project is to use a shared and harmonized protocol to address the central hypothesis that infections early in life lead to malnutrition by causing inflammation and/or by altering the intestinal barrier and absorptive functions, resulting in the failure of normal growth and development. A secondary goal of the project is to develop a collaborative network of investigators, institutions, and field sites as a platform for additional hypothesis-testing studies and interventional trials. Some of the existing companion projects are: genome-wide association study (GWAS), studies of the microbiome, improved diagnostics, studies of the immune response to polio and rotavirus vaccines, and studies investigating the relationship among breast milk, the microbiome, and the immune response. Dr. Lang indicated that there is potential for studies of mycotoxin exposure and health effects within the MAL-ED Consortium as companion projects. Some early data relevant to potential aflatoxin exposure include the age at which nuts and grains are introduced in the diet and the proportion of the diet that these foods represent. For young children, nuts or grains are often ground and put into porridges.

Discussion

In response to questions, Dr. Lang noted that children who have pathogens present, but do not have diarrhea can play an important role in disease transmission. Dr. Lang noted that the BMGF has a project on transmission of enteric pathogens. MAL-ED would like to do environmental sampling (food and water) in this regard. Dr. Lang stated that the MAL-ED project is looking at frequency of breastfeeding, number of feeds, and nature of weaning foods during the first 8 months of life; that is, the field workers ask about the number of times the child is breastfeed and about other food consumed. More quantitative measures of food intake are collected by 24 hour recall methods from 9-24 months of age. Dr. Ahmed commented that the Bangladesh site, as part of its case-control study, give malnourished children a full nutritional supplement; efforts are being made to keep track of what is eaten.

The various tests that are being done in the MAL-ED project have been standardized, field tested and validated. In the first year of the award, SOPs were developed and quality training was done. In terms of recruitment, field workers interview mothers in the third trimester. Healthy children are recruited between birth and 17 days of age.
In terms of sample volumes that might be available for mycotoxin measurement, 2 ml of plasma is frozen in
0.5ml aliquots, but this is the most precious and limiting of the clinical specimens collected and is largely
consumed by the existing protocol. It would be available from only some children, and in young children
may not be available at all. Urine is more plentiful, but usually only 2-3 milliliters are frozen for long term
storage. Normal and diarrheal stool, about 4 grams, are archived. These amounts are the goal; but variable
amounts are expected. The plan is to try to keep the samples for at least 4 years. The country of origin owns
the samples and maintains the archive; the data and samples can be shared among the MAL-ED
investigators. For investigators from outside the MAL-ED network, there would be a vetting process
including the requirement of joining the Consortium and abiding by its data sharing and other governance
clauses. At the beginning of the MAL-ED studies, a research consortium agreement (RCA), which outlines
these requirements, is signed by all participants.

The IRB consent form used by all sites states what the samples will be used for, but also states that the
samples could be used for projects beyond the objectives of the current study given additional IRB approval.
MAL-ED also has a bioethics advisory committee and a scientific advisory committee to provide advice to
the study. All protocols have to be approved by local site IRBs as well as the IRBs of collaborating
institutions in the U.S.

It was noted that although this is an observational study and not an interventional study, it is impossible to
prevent “messages” to be received by the study participants. This occurs at variable levels in different
countries. One of the reasons for including a limited case-control study was to control for any Hawthorne
effect.

Although it hasn’t been done yet, there are plans to determine if the data from an individual child can be
extrapolated to siblings, the home, and the community.

Dr. Ahmed stated that there is a project doing genome wide association studies looking at children in
Bangladesh who are not responding to malnutrition treatment. In studies of the SNPs associated with non-
response, three genes related to lipid metabolism have been found. Previous studies showed that levels of
essential of fatty acids in rural Bangladesh are among the lowest in the world. Efforts to replicate the SNP
finding in The Gambia and other sites are ongoing. Future studies would then look at the function of the
genes that are identified.

Field Studies

Role and mechanisms of aflatoxin in maternal anemia, low birth-weight and stunting among infants
and children in Africa
Pauline Jolly

Dr. Jolly reported on studies in central Ghana. She stated that anemia in pregnancy occurs frequently among
women in developing countries and is estimated to be the cause of 40% of all maternal deaths at childbirth.
Severe anemia can be as high as 40% among pregnant women. Anemia in pregnancy results in adverse birth
outcomes, such as, low birth-weight (LBW), small for gestational age (SGA), preterm delivery (PTD) and
stillbirths. 20 million infants worldwide (15.5% of all births) are born with LBW; 96% of these children are
in developing countries. Low birth-weight is closely associated with neonatal morbidity, inhibited growth
(stunting), impaired cognitive development, and chronic diseases later in life. Often, the anemia is present at
the time that the women become pregnant.

Dr. Jolly reported that 100% of women in Ghana who participated in these studies had aflatoxin in their
blood. There is chronic exposure to aflatoxins in the diet of Ghanaians and the aflatoxin B1 biomarker is
almost always present in the blood. In studies by others, aflatoxins have been shown to cause anemia in
animals (it is not clear if this is hemolytic anemia, which in animals is thought to be caused by toxins). Few studies have been conducted among pregnant women to describe levels of aflatoxins in blood, and health and birth outcomes, although publications in 1998 and 2002 reported that aflatoxin retarded fetal growth and resulted in LBW infants.

Dr. Jolly stated that there is a need for more directed research to evaluate the more subtle, yet probably significant, impact of aflatoxin on pregnancy and birth outcomes. The hypothesis of her work was that higher aflatoxin B1 biomarker levels in the blood of pregnant women would be associated with anemia as observed in experimental animals, and would result in higher levels of adverse birth outcomes. The specific aims were to: (1) examine the association between aflatoxin B1 (AFB1)-lysine adduct (AF-ALB) levels, and anemia in pregnant women in Kumasi, Ghana; and (2) examine the association between AF-ALB levels and birth outcomes among the women.

A cross-sectional study of 785 women presenting for delivery at two tertiary hospitals (Komfo Anoyke Teaching Hospital [KATH] and Manhyia Hospital) in Kumasi was undertaken. Aflatoxin test results were available for 755 (96%) women and were used in the analysis. The study included only women who had a singleton, uncomplicated pregnancy and whose anemia did not have a genetic cause. A questionnaire was administered to collect data on demographic characteristics and obstetric history for the current and previous pregnancies. Obstetric information was obtained from the women’s antenatal care charts. A 10 ml blood sample was collected for determination of: aflatoxin biomarker levels, hemoglobin and full blood cell count, folate, and malaria antigen. Stool samples were obtained for determination of intestinal helminths. The state of the newborn was determined at delivery by the midwives and included: live or stillborn, sex, weight and length.

Adverse outcomes were defined as: low birth-weight (LBW, less than 2500g), preterm birth (born before 37 completed weeks of pregnancy), small for gestational age (SGA, sex-specific birth-weight at or below the 10th percentile for the weight-for-gestational-age of an international reference population), or stillbirth (STB, born dead after 20 weeks of gestation).

Demographic data indicated that the average age of the women was 26.8 ± 6.3 years (range: 14-48 years); nearly half had a junior high school education, only 2.1% had a higher level of education; most women were employed and most were married; about a third were nulliparous; and a third had some type of flush toilet. In terms of the aflatoxin levels in their serum at delivery, the median was 5.0 pg/mg albumin, the mean ± SD was 10.9 ± 19.00 pg/mg albumin; and the range was 0.44 pg/mg to 268.73 pg/mg albumin. The participants were divided into quartiles based on aflatoxin levels: “low”: ≤ 2.67pg/mg, “moderate”: > 2.67 to ≤ 4.97pg/mg, “high”: > 4.97 to ≤ 11.34pg/mg and “very high”: > 11.34pg/mg. In terms of the health status of the mothers, 30% were anemic (Hb<11g/dL), 23% had iron deficiency anemia, 30% had low folate (3.9-18.1ng/mL), 17% were malaria antigen-positive, 23% had at least one helminth infection, with 16% having co-infection and 54.6% having neither infection.

Dr. Jolly summarized the statistical analysis and noted that there were not sufficient funds to do all of the desired analysis measures. Multiple logistic regression analysis was used to investigate the association between anemia (based on Hb <11g/dL) and aflatoxin levels. Variables that were statistically significant at P < 0.05 on bivariate analysis and those known to be associated with anemia based on extant literature were incorporated into models using the backward step-wise technique. The analysis for all 755 women was first conducted and then for the 580 of the women without a laboratory diagnosis of iron deficiency anemia (IDA). Measures of hemoglobin in red blood cells (RBC) were used as surrogates to identify women who may have had IDA.

Dr. Jolly summarized the findings as follows: The higher the AF-ALB level (quartile), the higher the percent of women with anemia (24.7% to 37.4%, trend p = 0.006 [Linear trend]). The dose-response relationship between AF-ALB quartile and increased odds of anemia remained after exclusion of the 175 participants with...
IDA. Stratification of the 580 women without IDA by malaria, intestinal helminth infection, and serum folate status, indicated a much stronger association of very high levels of AF-ALB with anemia among women with malaria or helminth infection.

Dr. Jolly described the implications of these findings as follows: With respect to the relationship of aflatoxin and anemia, the finding of increased odds of anemia with increasing levels of aflatoxin in blood is novel given that up to this time, there has been no study in humans showing this association. Based on previous findings in animals, these studies suggest that aflatoxin may cause anemia by inhibition or promotion of defective hematopoiesis, increased destruction of RBC, or by a combination of these mechanisms. It is possible that aflatoxin acts like most other toxins, and causes anemia by a hemolytic process. With respect to the association of maternal and infant anemia, several studies have shown that infants of mothers with iron deficiency are more vulnerable to iron deficiency and anemia. LBW and preterm infants are born with reduced iron stores and are at increased risk for IDA. Iron deficiency and anemia in young children adversely affects early childhood growth and cognitive development. With respect to the association between adverse birth outcomes and AF-ALB levels (n=755), based on previous publications, she hypothesized that higher maternal aflatoxin levels would be associated with low birth-weight and possibly other adverse birth outcomes. Bivariate analysis showed an increased risk of women in the highest AF-ALB quartile delivering babies who were SGA, LBW, preterm or stillborn; however, this was statistically significant only for LBW. Multivariable analysis also showed a significant association of high AF-ALB with LBW (OR, 2.09). Furthermore, when compared to women in the lowest AF-ALB quartile, there was a trend of increasing risk for low birth-weight with increasing AF-ALB levels ($P_{\text{trend}}=0.007$). Serum of mothers who delivered STBs had high AF-ALB levels (0.68pg/mg-229.49pg/mg albumin), but the association between STB and AF-ALB was not statistically significant (possibly due to the small numbers). With respect to aflatoxin exposure, LBW, and fetal and infant development, Mothers with higher AF-ALB were more likely to have LBW infants (aflatoxin possibly adversely affected fetal development leading to LBW babies). Exposure to aflatoxin continues after birth through breast milk and weaning foods. Aflatoxin suppresses immunity, making the infant prone to infections. Aflatoxin is associated with micronutrient (vitamins A, D, E, zinc, selenium) deficiency; these micronutrients are important for healthy immune system.

Dr. Jolly indicated that taken together these findings suggest that aflatoxin causes less efficient food conversion and decreases protein synthesis (The nutrition of these infants and children is already poor and along with the unsanitary environment in which they live results in frequent GI infections, that is, environmental enteropathy [EE]). Chronic local and systemic inflammation throughout childhood may be responsible for poor growth (stunting) of children.

**Discussion**

The following issues were discussed following Dr. Jolly’s presentation.

Dr. Jolly stated that she was not able to screen for hemoglobin “defects”. She was able to treat women with iron supplements, but she stated that even if women with the iron deficiency are treated, they still may have other causes for their anemia. It was noted that in addition to aflatoxin exposure, the poor diet of these women may be a cause of some of the anemia.

There was discussion about the difference in mycotoxin levels in the food available in the city markets versus that in rural communities, particularly if the farmers were sending their better products to market. It was stated that there is a perception that food in stores was safer than food in markets. There did not seem to be a lot of evidence at this point that there would be differences between a particular food in a rural setting versus in a town in the same general area. It was stated that the Centers for Disease Control and Prevention (CDC) has been looking at sources of maize in Kenya and found statistically significant higher levels of aflatoxin in home grown maize and that the most vulnerable population is subsistence farmers. It was also noted that in some cases farmers use “bad” maize to feed animals or to make other products (e.g., using nuts to make
soap); however, sometimes they eat it, especially in difficult situations such as famines. If was noted that if there are buyers who are known to value good quality grain and who will pay a higher price for it, farmers would likely be willing to sort out grains so as to take advantage of this.

The participants noted that there is a lot of country to country variation in terms of how the maize is marketed and in levels of contamination. In Guatemala, the maize sold in market place is maize that is grown in the lowlands, where production is easier and this maize has higher levels of fumonisins than maize grown in the highlands. In some countries, the maize that is sold right after the harvest is not very contaminated. There are regional differences in sorting maize and in tracing its origins. In Ghana, one can trace where the maize is coming from. In Kenya nothing is thrown away. Sometimes bad and good grain is mixed. In intervention studies in South Africa only about 2% of the maize was discarded. The study participants have a picture showing them the color of the maize that needs to be removed during sorting. In Guatemala, the maize is sorted while on the cob, with the three categories being: mixed, dirty and rotten. Some households will mix the corn 1/3, 1/3 to 1/3 and in that way, they wind up spreading out the dose over a year.

Mycotoxin exposure, impact on child health and intervention in African children

Yun Yun Gong

Dr. Gong stated that her work focuses on the use biomarkers to study health risk and intervention effectiveness. She defined exposure biomarkers as the chemical or its metabolites in biological samples that reflect the exposure level, measure the internal dose, can be directly related to effects, and are applicable to epidemiology studies on health effects and intervention studies. She noted that the advantage of biomarkers is that they reflect the internal dose rather than just food levels.

Using the AF-alb adduct biomarker, Dr. Gong presented comparative data on the levels of aflatoxin found in several African nations, in Brazil, and in the U.S. and Europe; with the levels in Africa being substantially higher. She also presented summary data of AF-alb levels in young children ages 6-16 months that have collected over many years in a number of countries in Africa. She noted that even within a country, there can be large differences over time, with age, and with season.

Dr. Gong provided an overview of the Aflatoxin B1 metabolic pathway and biomarkers. Hydroxylation of Aflatoxin B1 leads to several metabolites (AFM1, AFP1, and AFQ1) that can be detected in urine. Oxidation of aflatoxin B1 by CYP1A2, CYP3A4, CYP3A5 results in the Aflatoxin B1-8,9-epoxide. Detoxification of the Aflatoxin B1-8,9-epoxide by GST M1 leads to mercapturic acid conjugates which can be detected in urine. Hydrolysis of the Aflatoxin B1-8, 9-epoxide leads to the dihydrodiol which can be detected in blood as the Aflatoxin Albumin (AF-alb) adduct. Aflatoxin B1-8, 9-epoxide binds to DNA to form the DNA adduct (AFB1-N7-Guanine) which can be detected in urine. Through mutation, the DNA adduct (AFB1-N7-Guanine) can lead to the change in the TP53 codon 249ser that in epidemiological studies has been associated with hepatocellular carcinoma and can be detected in blood or tissue. Dr. Gong noted that one can select a variety of biomarkers from this pathway and use a variety of methods to detect and measure them.

Dr. Gong noted that there are few epidemiological data on fumonisins, mainly because of lack of biomarkers. Animal studies indicate a quick absorption rate and short half-life (few hours) for fumonisins. The majority of it is excreted unmetabolized in feces and a small amount in urine. FB1 is the primary form of fumonisins. It was previously detected in human urine using HPLC-F. LC-MS methodology allows for accurate measurement of it. SPE cartridges are used to clean up the specimens. Deuterium labeled FB1 (FBd6) is used as an internal standard for accurate quantification.

5 Aflatoxin exposure has been associated with specific mutations, notably a G to T transversion in the third nucleotide of codon 249 of the TP53 gene, referred to as the codon 249ser mutation.

6 FB1, FB2 and FB3 are the major naturally occurring fumonisins.
Dr. Gong presented data from studies testing the validity of the method for urinary detection of FB1 in a study of Mexican women. Maize tortilla consumption was used as a surrogate for exposure and urinary FB1 in the low, medium, and high maize consumption groups showed a good correlation with the detection of urinary FB1.

Dr. Gong noted that both aflatoxin and fumonisins have significant impact on human and animal health. In humans, aflatoxin has been linked to acute poisoning (aflatoxicosis), hepatocellular carcinoma (and possibly cirrhosis and other types of liver damage such as hepatomegaly), growth impairment and immunosuppression. An aflatoxin outbreak in Kenya in 2004 was a national disaster resulting in the death of more than 100 people from contaminated maize.

Although much of the data about fumonisins comes from animals, there is strong evidence of its toxicity as it has been shown to be hepatotoxic, nephrotoxic, carcinogenic in animals; fumonisins are acutely toxic to horses and pigs; fumonisins have also been shown to result in immune suppression. In humans, there has been linkage to cancer and neural tube defects (NTD). A recent study showed an association of FB with growth impairment in children.

Dr. Gong provided a brief review of data from aflatoxin and child health study in Benin. The key result was that aflatoxin exposure was associated with impaired growth in a cross-sectional study in 480 children. Using Z scores, there was a larger effect on height than on weight. In looking at exposure level, it increase from early age (less than two years) and then stabilizes to a more constant level when the children are older (between two years old and five years old). In a longitudinal study in which children were grouped into quartiles by exposure levels, and the adjusted height gain over eight months was measured, there were clear differences between children in the highest and lowest exposure quartiles. Attempts to measure the effects of several vitamins and micronutrients did not find a correlation to growth, but the work was not published and Dr. Gong felt that a large cohort study would be needed to prove any association.

Dr. Gong also reported the findings from a study in Kenya to determine whether aflatoxin exposure contributes to chronic hepatomegaly, a type of liver enlargement that is common in school aged children in Makuine, Kenya and that often co-occurs with splenomegaly (hepatosplenomegaly). In 2002, the hepatosplenomegaly prevalence rate in two neighboring village schools 2002 was around 80%. Multiple co-contributors to hepatosplenomegaly were present including malaria, schistosomiasis and aflatoxin. Additionally, the rates of schistosomiasis and levels of aflatoxin-alb adducts differed between the two villages. Dr. Gong noted that treatment for malaria did not change the rate of hepatosplenomegaly. In studies in 2002 comparing the mean AF-alb levels and organomegaly prevalence, there were differences in the levels of adduct among the different types of organomegaly, with hepatomegaly being associated with the highest levels of adduct. The presence of organomegaly was also indicative of adduct levels above those without organomegaly. It also appears that the occurrence of hepatosplenomegaly was multifactorial. However, it was not possible to determine if schistosomiasis was a confounder. Dr. Gong stated that the very high levels of hepatomegaly were seen only in 2002. In 2004, there was a decrease in the prevalence of malaria and of the splenomegaly, but not of the hepatomegaly. She noted that in 2004, there was an increase in the levels of aflatoxin-alb adducts.

Dr. Gong summarized ongoing evaluation of dietary exposure to aflatoxins and fumonisins in young children in Tanzania using urine and blood. She noted that the data are very preliminary. The focus was on maize consumption and on the need to control exposure to mycotoxins by educating mothers on food preparation and food storage. Studies are ongoing to evaluate the effectiveness of interventions that are focused on farming practices, particularly post-harvesting practices to improve the storage. The package that was used in Tanzania was an easily applied intervention had been used in a randomized trial in Guinea; in that case the intervention resulted in a 60% reduction in exposure level to AF-alb. In Tanzania, the focus was on FB, the intervention involved hand sorting and washing the grain and looking at the color of the grain to determine
what kind of water to use in the washing of it. The clean maize was cooked for consumption and used at a
two-day food party. The participants in the intervention were given training in the process.

At baseline, 86% of the participants’ intake of FBs exceeded the PMTDI of 2 μg/kg bw/day. FB1 exposure
was significantly reduced through the intervention whether measured as FB1 in porridge (mg/kg, 64%
reduction), FB1 Probable Daily Intake (μg/kg bw/day, 62% reduction), Urinary FB1 (pg/mL, 52% reduction)
or Urinary FB1 (pg/mg Creatinine, 41% reduction).

In terms of future plans, Dr. Gong suggested: enhanced international collaboration for more powerful
studies; studies of the mechanisms by which aflatoxins and fumonisins effect child growth, such as, by
epigenetic changes, by IGF-axis modulation, and by effects on gut function; studies of health effects caused
by aflatoxin alone or by aflatoxin/fumonisin co-exposure early in life; studies of the combined effect of
nutrient deficiency and infection on child growth; exploration of feasible and effective intervention
programs; and enhanced capacity building in Africa including educating of communities to promote
awareness.

**Discussion**

In terms of IGF1 assays, Dr. Gong stated that she uses an ELISA on serum samples and does not require
fasting prior to taking the serum sample.

There are different methods for measuring the levels of aflatoxin such as ELISA, LC-MS and fluorescence.
The amounts of aflatoxin by each method may differ as the various assays measure different metabolites;
however, there is general correlation of the findings. (ELISA measures a mixture of products, whereas HPLC
measures a single metabolite.) There is a method for back calculation of the results from these assays that
allows for the estimation of the amount of aflatoxin. For assays for aflatoxin only 250 ul of serum are
needed.

The data related to mycotoxins in the US are sporadic data. It was noted that products that are used locally
and are not involved in interstate commerce aren’t tested for mycotoxins. The data available suggest that
exposure levels in the US are low.

In terms of the persistence of the FB biomarkers and the potential for cumulative effects, it was noted that
fumonisin is a short term biomarker which might explain the lack of impact of fumonisins on long term
growth. It was also noted that there can be aflatoxin present in the area/event being studied which could
confound some of the findings about fumonisin. Alternatively, in conditions where aflatoxin is high, it
outcompetes the fumonisin.

A study of the kinetics of excretion of fumonisin in people eating tortillas in Athens GA showed that it peaks
on day 4 and by 72 hours post exposure. If there are more days of exposure, the half-life is stretched out a
bit, but the levels are still down to baseline in 96 hours. It is important to consider that biomarkers reflect
recent intake. In Guatemala, everyone consumes tortillas 7 days a week. In that situation, the FB1 levels
fluctuate, but persist.

In terms of whether fumonisin changes metabolism, it was stated that human disease is rarely studied. Tests
of liver function don’t show a large effect. However, the combination of aflatoxin and fumonisin could lead
to alterations in metabolism.

Fumonisin contamination is not a post-harvest effect; it is from contamination in the field and not from
storage.
There does not seem to be much data about the consistency of fumonisin contamination of maize, and about how it varies by country and seasonality. Samples from Guatemala that were taken three times a year (March, June, October) in the same three communities over a 10 year period showed that although there were some ups and downs, the levels were overall constant.

In terms of the hepatomegaly that is seen in Kenya there is only one paper that suggests that it is correlated to growth. It does not seem that liver function is damaged; although the hepatomegaly is chronic, it is reversible. With respect to the co-morbidities, it was noted that it is important to treat the person with schistosomiasis and not to just treat the water to remove the parasites. The presence of schistosomiasis exacerbates malaria, but itself does not seem to have a clear contribution to the organomegaly. Overall, the situation is complicated.

It was suggested that to better understand the hepatosplenomegaly in Kenya, hemoglobin electrophoresis could be done in a subset of children as there are a variety of hemoglobin diseases that cause hepatosplenomegaly. Whole blood would be needed for this and so an additional study would need to be done.

Concurrent Session, Group B: Understanding the Impact of Mycotoxins on Gut Function and Stunting Evidence, Potential Impact and Models

Is childhood stunting in Bangladesh due to exposure to aflatoxin?

*Tahmeed Ahmed*

Dr. Ahmed stated that Bangladesh has done well in reducing infant mortality. However, a significant problem is nutritional status; 43% of all children are stunted, including 8 million children under 5 years of age. A typical course of events in Bangladesh is for a 14 year old girl to marry and give birth to a low birth-weight baby in one year. 20-30% of babies are low birth-weight. Exclusive breast feeding has remained at about 40% level. Stunting is passed from one generation to another, with not only physical impairments, but cognitive impairments being part of a vicious cycle.

Dr. Ahmed stated that malnutrition in Bangladesh among children under 5 years of age is greater than that in many countries in Sub-Saharan Africa, although the perception is often that there is more malnutrition in Africa than South Asia. If one looks at the absolute number of children affected, the level in Bangladesh is the highest in the world. The population of Bangladesh has grown enormously in a rapid unplanned urbanization, with about 40% of the population living in slums. Dhaka is growing faster than Shanghai.

He stated that evidence-based interventions that show the most promise for reducing child deaths and future disease burden and could be implemented immediately include: breastfeeding promotion; appropriate complementary feeding; supplementation with Vitamin A and Zinc, and appropriate management of severe acute malnutrition. He noted that even if these interventions were implemented in 99% of children, stunting would only be decreased by 33%. Among the 10 other factors that likely contribute to the remainder of stunting are aflatoxin exposure and enteric enteropathy.

Dr. Ahmed noted that with respect to the prevalence of aflatoxin in Bangladesh, it is found in maize, groundnuts, and poultry feed at levels ranging from 79 to 480 ppb, with some poultry deaths being due to high levels of aflatoxin in the feeds. The percentage of positive samples in rice, pulses, and wheat ranges from 24 to 48% of positive samples. In a study of 96 healthy males, 49% had detectable AF-adduct levels, with the highest being 22.7 pg/mg. Bangladesh faces droughts and floods, both of which are conducive to aflatoxin transmission.

From a mechanistic perspective, metabolism of aflatoxin by cytochrome P450 3A enzymes results in an aflatoxin epoxide which can bind to DNA or and albumin. Gut enterocytes express CYP3A4, CYP3A5,
which is the site of aflatoxin activation to its toxic metabolite, and results in gut leakiness/increased permeability. This in turn results in malabsorption of nutrients and other effects, such as, LBW, chronic food insecurity, repeated infections. The end result of the process is stunting.

Dr. Ahmed showed the MAL-ED pyramid for the pathogenesis of malnutrition, the primary cause of child mortality and impaired cognition, and the potential cause of oral polio vaccine and rotavirus vaccine failures. The three corners are: (1) human genetic polymorphisms, controlling susceptibility to malnutrition, (2) diarrhea and enteric inflammation, compromising nutrient absorption and (3) the gut microbiome, enabling nutrient extraction from food.

Dr. Ahmed provided an overview of the MAL-ED project that is taking place in Dhaka. It involves a birth cohort and a case-control cohort as well as a discovery project. The discovery project includes a twin birth cohort, a twin case-control component, a pre-school cohort as well as an NIH ongoing birth cohort. Genome-wide association studies (GWAS) are planned as well as studies of the gut microbiome.

Dr. Ahmed showed a comparison of nutrition levels and illness events for two little girls, Moni and Papiya who were both 19 months old and lived in the Dhaka study site. Although over the 19 month study period, Papiya had more episodes of diarrhea, cough, fever, and ALRI, she was better nourished and less stunted than Papiya. The role (s) of enteropathy or mycotoxin in these stunting events is not clear.

Dr. Ahmed also noted that birth-weight is an important risk factor for malnutrition. Thus, there is a need to improve the nutrition of girls and women and to determine if women are more exposed to aflatoxin during pregnancy.

Studies of the micronutrient status of cases and controls showed that there is a high prevalence of anemia in all children (both cases and controls) as well as low levels of ferritin and high serum transferin.

In order to assess intestinal permeability, a drink containing a known ratio of lactulose:mannitol is administered. Both of these sugars are non-digestible sugars, i.e., they are not broken down/absorbed in small intestine. However if the small intestine is permeable, then these sugars will pass through the barrier and will be detected in a blood sample. The ratio of the sugars in the blood will reveal the type and extent of the permeability. Mannitol has the ability to pass through permeable cells (transcellular permeability). Lactulose cannot pass through permeable cells, but will pass through tight junction proteins if they become leaky and permeable (paracellular permeability). In the cohort that was studied, only 1/3 of children have a normal mannitol:lactulose ratio.

Dr. Ahmed described a proposed study to investigate aflatoxin exposure as a risk factor for stunting. The sites would be Dhaka (urban) and Trishal (rural) and a case-control design would be studied. The cases would be defined as HAZ <-2 and the controls as HAZ >-1. The marker of aflatoxin exposure would be aflatoxin-albumin adducts in venous blood and AF-N7-Guanine in urine. The estimated sample size is 150 cases and controls in each site (based on the levels prevalent in Africa and estimates of what these might be in Bangladesh). Dietary intake would be assessed by a food frequency questionnaire or 24 hour recall in a sub-sample. Morbidity data would be assessed by recall over the past 2 weeks. Aflatoxin is common many food items, such as rice, lentils, wheat flour. Dr. Ahmed stated that a lot of parboiled rice is used in Bangladesh (parboiled rice seems to foster aflatoxin as does the cycles of floods and droughts). Enteropathy would be assessed by the lactulose:mannitol intestinal permeability test, serum EndoCab antibodies, stool neopterin and myeloperoxidase, etc. Bivariate and multivariate analyses would be done.

Discussion

There was discussion about the kinds of mycotoxins that might be found in Bangladesh. It was stated that given the cycles of food and droughts, the large scale use and consumption of parboiled rice, and the
prevalence levels of stunting, it is very likely that aflatoxin is prevalent. In the India Rice Improvement Project, over 2000 samples were studied and 10-15% samples had high levels of aflatoxin. Another study showed that parboiled rice had high levels of aflatoxin. Additionally, the poor storage of rice is conducive to aflatoxin. Thailand has had a major post-harvest intervention for maize as has the Philippines. In Bangladesh, many of the safety net interventions include maize. Also poultry is one of the major sources of protein for middle class, and there is a lot aflatoxin in poultry feed.

It was noted that the mechanism by which the aflatoxin epoxide leads to a leaky intestine is not known. The assumption is that the aflatoxin is causing the damage at the enterocyte level and there is also some evidence related to tight junction formation. Because the epoxide reacts chemically with albumin, it has the potential to cause tissue damage. The epoxide also reacts with histones which can cause changes in gene expression.

It was noted that in some of the older literature, when there was a slight Vitamin A deficiency, aflatoxin was more carcinogenic. It is also important to consider that if one is looking at a population that is immunocompromised, there are changes in phosphorylation and hence mechanisms of action.

In terms of the lactulose:mannitol tests in the MAL-ED, there was some site-to-site variability with known standards that likely reflects differences in equipment. While this makes site-to-site comparisons difficult, within a site, the results should be internally comparable. Of the 8 countries participating in the MAL-ED project, 4 facilities are doing the assay and a lot of QC activities have been undertaken in the last year. It was noted that there is a clinically standardized protocol for Irregular Bowl Disease, and using this clinical protocol might be helpful. It was also remarked that the assay does not discriminate between persons who are malnourished and those who are not. Dr. Ahmed stated that there are also longitudinal samples for his cohort that are being analyzed.

Mannitol is a measure of the absorptive capacity of the intestines. The lactulose:mannitol ratio is a function of leakiness. It was noted that the environmental enteropathy also results in reduced surface area in the intestines. Although there are measures for the changes in permeability, the reduced surface area can also be an additional problem and it was not clear if there is a way to measure this. Calprotectin\(^7\) was proposed as another potential biomarker of intestinal damage and pilot studies using it are ongoing.

The occurrence of diarrhea and the transmission of diarrhea-causing organisms are difficult to explore through case-control studies which are measuring only one point in time. There is not information in these kinds of studies of recent diarrheal episodes. Asymptomatic colonization by diarrhea-causing infectious agents also needs to be considered.

It was stated that there are ongoing studies of the gut microflora through routine microbiology and multiplex PCR. There are also studies using metagenomics to assess the microbiome, which may provide some insights. The microbiome may be related to the availability of different micronutrients or may be related to malnutrition.

It was noted that within the Bangladesh cohort, the investigators are looking for “positive deviants,” that is looking at children who are doing well and determining what their parents are doing. In the case of the two 19 month old girls that were reported on, the child that was doing better had more animal source food.

\(^7\) Calprotectin is a 36kDa calcium and zinc binding protein that accounts for 30 to 40% of neutrophils' cytosol. It is resistant to enzymatic degradation, and can be easily measured in feces. Measurement of fecal calprotectin has been used as a biochemical test for inflammatory bowel disease (IBD).
It was noted that in India and Bangladesh, the liver cancer rates are low. There is a robust immunization program with all children getting the HBV vaccine. However, the national program does not measure antibody titer. It was stated that in India, there is about a 4-6% carriage prevalence and the vaccine is close to 100% effective.

**The effect of different mycotoxins on the intestinal barrier function and on the immune response**  
*Isabelle Oswald*

Dr. Oswald stated that her interests are on the effect of mycotoxins on the immune response, the effect of mycotoxins on the intestinal barrier, and strategies to limit mycotoxin contamination/effects. The mycotoxins of interest are aflatoxin, fumonisin and deoxynivalenol (DON). The working hypothesis is that mycotoxins affect intestinal functions and the systemic immune response. The effects on intestinal functions involve effects on proliferation physiology, and thereby, on nutrient adsorption; effects on barrier function, and thereby, on passage of pathogens and toxicants; and effects on inflammation, and thereby, leading to inflammatory disease. The effects on the systemic immune response lead to susceptibility to infection and vaccination failure.

Dr. Oswald stated that the pig is a very good model for human disease as the pig is very sensitive to mycotoxins and there is a similarity between the pig’s intestinal and immune responses to those of humans.

A vaccination model has been developed using piglets. The piglets are exposed to mycotoxin for four weeks. They are vaccinated during the first week of dietary exposure to mycotoxin and boosted during the second week. Measures include: cell-mediated and humoral immune responses, antigen-specific and total immune responses, and regulation by cytokines (protein and mRNA responses). An infection model has been developed in piglets and involves oral infection with pathogenic strains of *Escherichia coli*.

Dr. Oswald also described the three levels of the piglet model system that are used to investigate effects on the intestinal barrier. The *in vivo* model uses experimental feeding with contaminated feed which allows for an exact amount of toxin exposure. The *ex vivo* model uses intestinal explants (biopsy punches) that are exposed to toxins. This system has a limitation to 150 biopsy punches as there is a need to take the cells from the animal to the incubator in one hour. The cellular model uses intestinal epithelial cell lines (IPEC-1) exposed to toxins. This system uses a non-transformed cell line which is more sensitive to toxins than transformed cell lines. Analyses with these three systems examine three areas: (1) intestinal morphology (crypt/villi length, lesion score), (2) intestinal functions (cell proliferation/renewal, barrier function, immune response, and (3) mechanism of action (junction proteins, MAPKinase, sphingolipids).

Dr. Oswald summarized studies with each of the mycotoxins using the different model systems.

Studies with DON showed that it decreases intestinal cell proliferation in the three models. The *in vitro* data show a decrease in cell proliferation which is dose-dependent. The effect is also seen *ex vivo* when the explants are treated for 4 hours and *in vivo* when the piglets are treated for 4 weeks. The studies use piglets that are just weaned, and because of their small size, less mycotoxin is needed. Histological studies of explants or of intestinal tissue from treated animals also showed alterations in the intestinal tissue and as a consequence a decrease in weight of DON treated animals compared to controls was observed.

Studies in which electrodes are placed on the apical and basal parts of cells, demonstrate that DON decreases trans-epithelial electrical resistance (TEER). This type of cellular alteration could lead to increased passage of pathogens or toxicants. This increased trans-epithelial passage was demonstrated by *ex vivo* studies showing increased passage of fluorescent dextran and by *in vitro* studies showing a dose-dependent increased passage of bacteria across the intestinal monolayer.
Additional studies were undertaken to determine the mechanism by which DON acts. *In vitro* studies using fluorescent staining showed that DON decreases the intestinal expression of the claudin proteins. *In vivo* histological studies and Western Blots also found the same result. Further studies using an inhibitor showed that DON decreases the barrier function by altering the claudin expression through MAPKinase activation.

Studies examining the immunological effects of DON have shown that DON induces a Th17 intestinal inflammatory response.

Dr. Oswald stated that with respect to DON’s effects on the intestine, there is a need to: (1) understand the relationship between the intestinal immune response and the alteration of intestinal barrier function and (2) determine the consequences of this in terms of susceptibility to enteric infections.

Dr. Oswald summarized studies on the effects of Fumonisin B1 (FB1) with respect to impairing the immune response to vaccines using the piglet model. She noted that the decrease in response is seen in the specific antibody response, but not in total antibody. It has been observed when vaccinating against mycoplasma and also with the response to ovalbumin. In studies examining cytokines, the data show that FB1 impairs the vaccine antibody response through an alteration of the Th1/Th2 cytokine balance, both *in vitro* and *in vivo*. There is a decrease in IL-4 (Th2) and an increase in IFN-γ (Th1).

Dr. Oswald also showed *in vitro* data that FB1 alters trans-epithelial resistance and results in an increase in mycotoxin passage. She stated that FB1 alters intestinal tissues with consequences on gut physiology as evidenced by increased lesions in the jejunum and ileum. Studies examining absorptive and secretory responses show an increase in glucose and a decrease in phlorizin. She noted that this type of altered gut physiology is reminiscent of what is observed with other stressors, such as, food deprivation or change, or weaning. *In vitro* and *in vivo* studies also showed that FB1 decreases intestinal expression of IL-8, both at the mRNA and protein level. Dr. Oswald noted that the immunological implication of this decrease relates to the fact that IL-8 is implicated in the recruitment of neutrophils during an inflammatory response so that decreased IL-8 production could lead to an impaired recruitment of neutrophils. Although neutrophil levels were not measured, *in vivo* studies in piglets showed that decreased intestinal IL-8 synthesis (ileum, caecum, colon) was associated with an increase susceptibility to enteric infection with E. Coli (given by the oral route). Dr. Oswald stated that there is a need to further characterize the effect of FB1 on the intestine, especially on the intestinal immune response.

Dr. Oswald noted that since all three of the mycotoxins she studied had effects on the immune response, it is likely that they also were involved in the response to vaccines. She presented data using the vaccine model in piglets and measuring the response to an ovalbumin vaccine. She noted that aflatoxin did not affect the antigen-specific humoral (antibody production) response, but did reduce the antigen-specific cellular (lymphocyte proliferation) response. Based on the literature and her experimental results, she proposed a mechanism by which this might occur. Aflatoxin triggers an inflammatory response, especially IL-6, resulting in increased expression of inflammatory cytokines in the spleen of mycotoxin exposed animals. In mice, IL-6 has been shown to maintain dendritic cells in an immature state. In this way, IL-6 decreases the antigen-specific lymphocyte proliferation without affecting mitogenic proliferation. Thus, while in FB the effect is on the antibody response, with aflatoxin, the effect is on CMI, but not on antibody.

Dr. Oswald stated that there is a need to investigate the effect of AFB1 and AFM1 on the intestine, both on the immune response and barrier function.

**Discussion**

Dr. Oswald stated that it is hard to find animal feed that is not slightly contaminated and that co-contamination is the rule and not the exception. She noted that there is a need to characterize the effect of mycotoxins on intestinal microbiota.
Several points were made about features of the piglet model. Piglets provide the ability to do stem cell studies and also the ability to look at differential effects along different part of intestine. There is also the potential to look at the effects of dietary modifications. There is a need to determine if the pigs have the appropriate enzymes, for example for metabolizing DON. Although the mycotoxin dose in the model is higher than the dose that the piglets would be exposed to in a farm situation, Dr. Oswald stated that it is important to have a dose that consistently yields a clear effect because only small numbers of animals, 5 to 6, per experimental group can be used. It would be difficult to do studies using sows exposed to mycotoxins and measuring the effects on the piglets, as very large amounts of mycotoxins would be needed because of the large size of the sows. There is a need to use a physiologically relevant parasite in the TEER studies, that is, a parasite that normally translocates in the intestine.

In terms of the relationship of immunological effects measured in the gut to systemic effects, it is likely that impairment of the gut immune system would reflect effects throughout the body, although there is the potential for separate effects. In this regard, in a study in humans in Africa, effects on the sIgA in saliva were seen. In response to OSHA’s interest about the effects of worker exposure to grain dust and aflatoxin, a study was done in rats using nasal inhalation. The investigators found both systemic and lung-related pulmonary and macrophage effects that lasted for a number of weeks. Dr. Oswald noted that she had done studies in which fumonisin was given orally and she observed increased susceptibility to nasal infection by Pasteurella. The measure of infection in that study was a count of the number of times the pigs coughed. Together, these studies demonstrated that that intestinal exposure to a mycotoxin can have systemic consequences on the immune system.

A study in West Africa looked at T cell subsets; some subsets were suppressed and lower levels of cytotoxic T cells were consistently found. Thus, it may be important to look at T cell subsets when assessing immune effects of mycotoxins.

It was noted that the phenotypic responses to mycotoxins can be different in different people, in this respect, a genomic approach looking at patterns of genetic change might provide some mechanistic insights. One could look at the regulation of genes related to the immune response to determine if there is pattern related to mycotoxin exposures. Longitudinal studies would be relevant in this regard as the recentness of the exposure could have an effect. Alternatively the use of animal models would allow for timing/dosing of the exposure. Another point of view was that just finding up or down regulation is not enough as it does not provide insight about the health outcome(s); thus, it might be better to just measure the direct health effect, e.g., occurrence of diarrhea. However, it was noted that there are many factors that can induce a health effect such as diarrhea or coughing.

Stunting is an effect that basically occurs in the first two years of life. The biomarker in the blood may still increase, but the stunting stops. It was noted that in a study in Benin, stunting did not stop at 2 years of age, but continued to ages 3 through 5; in that study, the exposure to aflatoxin was increasing along with the age of the child. Studies from The Gambia suggest that the biomarkers in children don’t increase beyond the level that is found in adults.

It was noted that animals can adapt to the mycotoxin in that the change that induces the stunting could also the animal’s metabolism. Moreover, the impact of a mycotoxin on a growing animal is higher than the impact on older person.

It was noted that there is underlying inflammation from the different infections that individual people are exposed to; the mycotoxins are just another danger signal that the cells respond to. Once cytokines are produced in response to a danger signal, there is a “sickness” immune response.
It was stated that in radiation biology, one studies the sensitivity to radiation in terms of stage of development of the exposed individual. The two major waves of DNA synthesis are found in the very young and during the teenage years. In case of animals, those exposed to radiation pre-weaning don’t get tumors, but those exposed after weaning do get tumors. In this regard, in children the “terrible twos” are a time of neural pruning which is why the toxic effects of lead in children are seen at age 2. Thus, one needs to look at effects in the context of the organism.

Mycotoxin Liver Injury in Children: Regenerative Hepatobiology as a Model for Human Growth Failure from Mycotoxin Exposure

David Rudnick

Dr. Rudnick stated that his presentation would focus on five areas: (1) mycotoxins and hepatotoxicity, (2) chronic liver disease and growth failure, (3) a hypothesis that chronic liver injury contributes to stunting in mycotoxin-exposed children, (4) mechanistic considerations related to this hypothesis involving lessons from regenerative hepatobiology and from the growth hormone IGF1, and (5) a model of hepatic involvement in mycotoxin-associated stunting.

Dr. Rudnick noted that there are considerable data in both animals and humans linking mycotoxins and liver cancer. The animal data include the effects of chronic exposure to aflatoxin and other toxins, have been done in a range of animals including rodents, nonhuman primates, fish, fowl, and have shown a dose-dependency of effects and of the existence of genetic and environmental modifiers. In rodents, partial hepatectomy increases the risk of tumors.

Human data linking mycotoxins and liver cancer include case-control studies showing genetic susceptibility and cohort studies showing the etiologic role of aflatoxin in HCC and showing chemical (aflatoxin) and viral (HBV) interactions.

Dr. Rudnick showed examples of animal data demonstrating mycotoxin-induced hepatotoxicity in a range of animal species, involving exposure to penicillium-, rubra-, and afla-toxins and involving injury to the liver as demonstrated by serum transaminase levels and histopathology. He showed data from animal studies demonstrating the specific types of histopathological characteristics of acute hepatotoxicity (hepatic steatosis, centrilobular necrosis), subacute hepatotoxicity (centrilobular necrosis, fibrosis, bile duct proliferation), and chronic hepatotoxicity (fibrosis, cirrhosis, cancer). Dr. Rudnick noted that any liver injury associated with cirrhosis increases cancer risk.

Dr. Rudnick cited publications related to mycotoxin-induced growth failure in animals and in humans, which raises the issue of whether liver injury mediates mycotoxin-induced growth failure. In this regard, he noted that stunting is seen in children with End Stage Liver Disease (ESLD) who are awaiting a liver transplant.

Dr. Rudnick stated that there are three candidate mechanisms that could be assessed to test that hypothesis that chronic liver injury contributes to growth impairment in mycotoxin-exposed children. He presented data related to each of these.

The first mechanism relates to inadequate caloric intake or nutrient malabsorption as contributing to growth impairment. Dr. Rudnick stated that Dr. Jolly’s data was supportive of this. The second mechanism relates to catabolic response to liver injury. This model involves partial hepatectomy, and monitoring the liver as it grows back over time; the liver continues to grow until the original ratio of liver to body weight is reached. Dr. Rudnick noted that early after the surgery there are profound hepatic metabolic and systemic effects: there is an effect on fat accumulation; this fat accumulation follows the hypoglycemia that is seen right after surgery. There is also evidence of systemic catabolic response to partial hepatectomy. This kind of catabolic
response also occurs after carbon tetrachloride insult and could also occur in response to aflatoxin liver injury.

The third mechanism involves impaired hepatic growth factor production (IGF1), particularly with respect to resistance to growth hormones (GH). Dr. Rudnick stated that Dr. Petska has published data about impaired hepatic growth factor (IGF1) production. IGF1 is made in the liver and is the primary effector of growth hormone effects. It appears that IGF1 and IGF2 levels aren’t affected, but rather that GH resistance occurs. (This is also seen in end-stage liver disease.). The potential mechanisms by which this may happens are that TNF-α suppresses hepatic GH receptor expression or that IL-6 suppresses hepatic GH receptor signaling possibly through suppression of STAT5 activation. Dr. Rudnick presented findings related to these two potential mechanisms. He indicated that a catabolic response is observed, for example, in animal studies in which the bile duct is ligated (BDL); there is a loss of weight, of fat free mass and of fat mass. In animals with BDL compared to controls, there is a reduction in hepatic IGF-1 mRNA, hepatic GHR protein and hepatic pSTAT5. Dr. Rudnick stated that cytokine and growth hormone signaling are required for liver regeneration, including TNF-α, IL-6, and GH signaling. He noted that acute liver injury of any kind leads to a catabolic response as well as regenerative signals. The regenerative response leads to suppression of the catabolic process. However, chronic liver injury leads to impaired growth as well as sustained catabolism.

Dr. Rudnick felt that the data that he presented support a model of liver-dependent mycotoxin-induced stunting. In this model, acute liver injury (e.g., by partial hepatectomy, carbon tetrachloride, aflatoxin) leads to both a metabolic (catabolic) response and to regenerative signals (IGF-1, GH). The regenerative signals would normally shut down the catabolic response as well as lead to growth. However, in the face of chronic liver injury, the signaling for shutting down catabolism as well as the signaling for growth would be impaired. He suggested that this model for growth impairment could be tested by assessment of liver injury and function. Some of the ways that this might be done include: measuring the extent of detectable liver injury and dysfunction in at-risk individuals and populations; (2) determining if liver assessment be exploited to assess or quantify toxin exposure in populations, (3) using analyses of toxemic hosts to illuminate liver-dependent growth consequences of mycotoxin exposure.

Discussion

Given its function in the body, the liver is a target organ for aflatoxin.

Regarding data linking end stage liver disease with growth impairment in children, it is possible that the underlying cause of the liver disease (often genetic) also contributes to stunting in ways that are independent of liver injury. Nevertheless, published data shows that improved growth after liver transplantation is possible, with children under 2 years of age demonstrating greater “catch-up” than those aged 2-7 years. That observation suggests that liver injury itself is an important contributor to growth impairment.

A question was asked about effects on children of maternal alcohol consumption during pregnancy. Fetal alcohol syndrome is characterized by stunted growth as well as neurocognitive abnormalities and characteristic physical findings. A question was asked about whether the catabolic response to toxin-induced or other forms of liver injury might be harnessed for therapeutic advantage, for example in the setting of obesity. Dr. Rudnick did not think this would be either safe or possible. The role of growth hormone (GH) in the context of stunting associated with liver diseases was further discussed. There is some evidence to suggest that liver and other inflammatory diseases cause hepatic resistance to growth hormone activity, which might limit the potential benefit of GH in these diseases. Nevertheless, one study of children with inflammatory bowel disease, which also leads to GH resistance, reported some modest benefit of GH.

Some participants felt that fatty liver disease is a factor that is driving liver cancer, although others felt that this linkage was not clearly demonstrated. If there is this association, then it is possible that in the over-
nutritioned populations in the US, more liver cancer will be seen. In Bangladesh, arsenic and other factors could be the drivers of liver cancer in terms of creating a tipping point.

In terms of translating the findings in animal models of stunting to humans, the issue is complex as stunting is multifactorial and it is more than the pharmacology of the toxin and the target of the toxin.

With respect to chickens as a model for stunting, it was noted that growth rate is very important as chickens are put into market at about 14 weeks of age and a farmer’s profit depends on the animals’ growth. Any farmer with a decreased growth rate will test their feed. Chickens have a normal life span of 20-25 years.

February 2, 2012

Concurrent Session, Group B: Understanding the Impact of Mycotoxins on Gut Function and Stunting: Mechanisms and Biomarkers

Aflatoxin and growth faltering: potential mechanisms
Paul Turner

Dr. Turner reiterated that chronic exposure to mycotoxin can have deleterious effects on health. Aflatoxin B1 has been associated with liver cancer, liver/kidney toxicity, immune suppression, and stunting of growth of children. Fumonisin B1 has been associated with esophageal cancer, neural tube defects, and possibly with effects on growth. Deoxynivalenol (DON) has been associated with gastroenteritis, immune modulation, vomiting and in animals models with effects on growth.

With respect to growth faltering, an estimated 33% of children in West Africa have stunted growth. Diet and infections are critical components that contribute to the burden of growth faltering in some of the least developed regions of the world. In parts of West Africa, over 40% of the observed growth faltering during the first year of life has been linked to damage to the intestinal mucosa. Growth faltering is not fully explained by dietary insufficiency and infection. Dietary supplementation doesn’t restore the faltering and exposure to toxins does not account for all of the deficits in growth.

Much of the developing world is at risk for exposure to aflatoxin, with Africa and South Asia being at the highest risk. Dr. Turner has focused his studies on aflatoxin in West Africa where maize and peanuts are the major sources of contamination. Diets in the regions where aflatoxin is a risk are quite boring, that is, the population has access to a limited variety of food. Over the past 30 years, a number of aflatoxin biomarkers have been developed that are based on the principle metabolites of aflatoxin and these biomarkers have been used to study blood and urine specimens.

Dr. Turner summarized work done in Benin and The Gambia, areas that have a high frequency of exposure and large ranges of exposure. In a cross-sectional study in Benin, exposure to aflatoxin was associated with reduced growth, and larger growth deficits were associated with higher amounts of AF-alb. A cohort longitudinal study also showed a dose-response relationship in reduction of growth velocity and AF-alb levels.

In Gambian children aged 6 to 9 years of age, growth faltering was modest in relation to AF-alb. These children have chronic exposure to high levels of aflatoxin. A scatter-plot of AF-alb versus HAZ is suggestive of a cut-off point of approximately 30pg/mg, with values less than that not showing a dose-response and values beyond that showing more of a dose-response. Dr. Turner noted that these data were suggestive at this point, but could imply that there is a threshold for effects. If there is a threshold, it might be different for different age ranges.
Dr. Turner then addressed a potential mechanism for the growth faltering in Gambian infants. If weight gain and intestinal permeability (as measured by lactulose:mannitol) are plotted on the Y-axis of a graph with age of children (0-16 months) on the X-axis, although each Y-axis factor may vary up and down with time, the lines are a mirror image of each other; that is, as intestinal leakiness goes up the weight gain goes down. Likewise separate plots of weight-for-age (WAZ) and HAZ show declining slopes when the lactulose:mannitol ratio increases.

In terms of exposure in utero, Dr. Turner stated that aflatoxin can be transmitted across the placenta. In a study in The Gambia, 140 women were studied during pregnancy and there was follow up for 1 year after the birth of the infants. Measures included anthropometry at intervals from birth to week 52 and blood samples for AF-alb from maternal blood, cord blood, and from infants at week 16 and week 52. Dr. Turner stated women were exposed to aflatoxin at high levels during pregnancy. The aflatoxin biomarker levels in utero suggested that the aflatoxin was in the activated form. By 16 week of age, 20% of the children had evidence of aflatoxin exposure; by 52 weeks of age, children had the same levels of AF-alb as adults. There was also seasonal variation in exposure, reflecting differences in food just harvested and food that was in storage. Dr. Turner reported the results of the GEE multiple regression analysis that was used to assess the effect of maternal AF-alb on WAZ or HAZ. Maternal AF-alb was associated with WAZ score and with the HAZ score, all of which were adjusted for sex, age, placental weight, maternal weight, gestation duration and season. The analysis predicted that a reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg would lead to a 0.8 kg increase in weight and 2 cm increase in height within the first year of life. Week 16 AF-alb additionally contributed to the model for HAZ though not for WAZ. Dr. Turner stated that taken together these findings stress the importance of early intervention.

In terms of mechanisms for maternal AF-alb, Dr. Turner stated that maternal intestinal damage is not likely unless the mother has poor nutrition. Other alternatives include liver toxicity, Zinc bioavailability, maternal toxicity and placental toxicity. Dr. Turner stated that results from pigs show that Zinc bioavailability in breast milk not a problem, but never the less there could be an effect from reduced Zinc bioavailability on Zinc carrier proteins, which could result in effects both on the immune system and on growth.

Dr. Turner proposed a number of issues that need further exploration. There needs to be a definition of the critical time points in the perinatal period for the adverse effects of aflatoxin exposure on child growth in West Africa. No studies have measured exposure through pregnancy and into the first few years of life. No studies have measured intestinal damage, aflatoxin, and growth in cohorts.

Aflatoxin suppresses the immune response and increases susceptibility to infections in animals and there are reports of associations between aflatoxin exposure and immune dysfunction or disease susceptibility, and of associations between aflatoxin exposure and anemia. There needs to be exploration of whether the MAL-ED cohorts can investigate mycotoxin-infection interactions or whether larger cohorts or novel cohorts in high risk settings are needed to investigate this issue.

Dr. Turner stated that the underlying mechanisms of aflatoxin associated growth impairment in early infancy need to be elucidated. Possible mechanisms include: altered intestinal permeability, impaired nutrient uptake, compromised (intestinal) immunity, and liver toxicity (possibly related to IGF1). An important issue that needs to be addressed is whether intervention approaches to restrict aflatoxin exposure can alleviate mycotoxin exposure of infants and restrict growth faltering. This kind of intervention study may provide strong causality data and promote more global intervention strategies.

There is a need to decide on which type of intervention to use, primary prevention, uptake inhibitors, or chemopreventon. High tech interventions are not useful in the areas that are most affected by mycotoxin contamination of food.
Dr. Turner provided an overview of an intervention study in Guinea. This involved different methods of ground nut drying, the use of hand sorting, the use of a variety of types of bags, the use of a pallet for storage and the use of insecticide in the storage area. It involved 300 people and the results showed a 55% reduction in the individual AF-alb levels. Dr. Turner noted that there is also the potential for combinations of interventions.

For future work, Dr. Turner proposed: (1) understanding the mechanisms of growth faltering and interactions between aflatoxin and additional factors, including the support of targeted interventions and developing an understanding of critical exposure windows and exposure thresholds; (2) examine the global contribution of aflatoxin to growth faltering. The BMGF are supportive of this type of activity. This kind of work would involve support of targeted interventions, establishing and involving at risk communities in education programs to restrict exposure, extending to maize the Guinea study on the ground nut crop, the use of radio broadcasts on local agricultural stations and community education activities in Egypt.

**Discussion**

There was discussion as to whether all three major mycotoxins or others should be studied at the BMGF-supported sites. It was suggested that there needs to be an assessment on a site-by-site basis to establish if there was risk related to specific mycotoxins. It was stated that Bangladesh is appropriate for studying aflatoxin; FB is more prevalent in southern Africa. DON has been found in crops in India and Brazil. It was noted that a good biomarker for DON has just been found. A small scale pilot study might be the best way to assess the issue of multiple mycotoxins in a particular dietary staple. There are no data as to whether the very high levels of mycotoxins or the chronicity of exposure is driving the health effects that are observed.

In response to a question as to whether there are data about adducts in terms of the adolescent growth spurt, it was suggested that there might be some in studies in The Gambia. It was noted that in the study in Kenya with children 7 to 14 with hepatosplenomegaly, there did not seem to be a growth issue. It was stated noted that in many of the older studies, IRBs would not allow for study of persons under 18 years of age.

It was stated that in NE Brazil there was a decline in stunting over a 10 year period as a result of maternal education and an increase in purchasing power. With increasing wealth, people are able to eat a more varied diet and so the exposure to aflatoxin may have decreased. There is frozen blood from this period, 1996-2006, that may also yield data on exposure.

With respect to early interventions in children to prevent stunting, the question was raised as to the feasibility of the introduction of more dietary variety in the weaning period. It was stated that it might be feasible in some communities at some times. There is a need to visit each site and examine the agroecology. Dietary variety would reduce exposure and also provide micronutrients. However, a large educational effort would be needed and it may not be agriculturally possible in some areas to grow or to have access to a variety of foods. It was stated that the agriculture team at the BMGF had looked at the issue and felt that fortifying some of the stable foods rather than horticultural change might be more feasible. It was noted that dietary sufficiency is the goal in most places and that excess agricultural space is often used to raise for crops for sale.

In terms of aflatoxin getting into human milk and its implications for breast feeding and recommendations for exclusive breast feeding, it was stated that only 1-3% of the mother’s aflatoxin dose is transmitted into the milk. This is an effect of the pharmacodynamics of excretion. Breast feeding also helps with immune system development. Once the child eats plate food then exposure to aflatoxin can be hundreds of times higher. An additional factor related to storage depots in the body for mycotoxins, is that a lot of mobilization of fat depots occurs during pregnancy and lactation. Since aflatoxin is stored in fats, the first borne child might be at a slightly higher risk than subsequent children. However, while aflatoxins are lipophilic, DON is not found in fat depots nor is FB1; FB2 has a different solubility than FB1.
It was stated that seasonality may affect maternal exposure. However, there may be regional differences as to whether there are seasonal effects for exposure. Additionally, the storage processes for different staples, such as maize and ground nuts, are different. The same crop may also be stored differently or for different periods of time in different regions or countries.

It was noted that there are ongoing clinical trials in Malawi and Ghana supported by the BMGF that are enrolling women during pregnancy. Although these trials are not designed to address mycotoxins, there may be an opportunity for companion studies.

With respect to variations of growth factors in different age groups, it was stated that IGF1 does not appear to be critical for early infant growth. However, there is little information in this area.

**Human Toxicology of Aflatoxin and Potential Impacts in Micronutrient Deficient Populations**  
*John Groopman and Kerry Schulze*

Kerry Schulze focused on micronutrient trials in Asia. Most of these trials were done in the 1970’s and early 1990’s and provided evidence that micronutrient status (vitamins, anti-oxidants, trace elements) affects aflatoxin toxicity, and that micronutrient deficiencies can make the gut more susceptible.

She cited the Nepal Nutrition Intervention Project, which included a randomized clinical trial (RCT) of Vitamin A and beta-carotene supplementation in women of reproductive age (1994-1997) and an RCT with 5 arms of antenatal micronutrient supplementation regimens (1999-2001) in which children were surveyed at 6-9 years of age (2006-2008). Dr. Schulze also cited the JiVitA Project in Bangladesh which included an RCT of antenatal Vitamin A and beta-carotene supplementation (2003-2007) and an RCT of antenatal iron-folate versus multiple micronutrient supplementation that is ongoing. She noted that since these trials focused on pregnancy and the participants are being followed throughout life, it is possible to assess the current health status. Additionally, there are some materials left from these trials that could be used for other types of studies.

The Nepalese population is quite micronutrient deficient, including deficiencies in Zinc, Iron, and vitamins as well as having a high prevalence of anemia. Of women studied during early pregnancy, 33% had anemia, 40% had IDA, about 15% had inflammation, and more than 95% of the women had at least one deficiency. Of children between 6 and 9 years of age, about 25% are anemic, about 70% have adequately iodized salt available, about 55% have at least one deficiency and about 33% have concurrent inflammation.

Observations of Bangladeshi women in early pregnancy demonstrated Vitamin A deficiency and lower rates of anemia (20% versus 33%) than in Nepal and with none of it related to Iron deficiency; there is less Zinc deficiency (15% versus 61%), but more iodine salt deficiency (70% versus 50%). About half of the children are low birth-weight.

Dr. Schulze cited findings from several intervention studies. Studies of main effects showed that Vitamin A and beta-carotene reduced maternal mortality in Nepal and that Iron, Folic Acid and multiple micronutrient antenatal supplementation improved birth-weight by about 60 grams compared with a control population that received Folic Acid, Iron and Zinc. Five other studies of longer-term benefits of *in utero* micronutrient exposure include: (1) a 31% reduction in childhood mortality at 6 to 9 years of age in the Iron-Folic (FE-FA) Acid Group; (2) an improvement of cognition with Fe-FA; (3) a 0.64 cm increase in growth with FA-Fe-Zn; (4) Lower metabolic syndrome risk factors with FA (5), improvement of lung function with Vitamin A.

To assess the effect of aflatoxin on these benefits, the samples from the children 6 to 9 years of age were studies as well as the aflatoxin levels in their mothers during the first and third trimesters. When children were grouped by Z scores, the deficit in height-for-age and weight-for-age correlated inversely with the level
of aflatoxin adduct. In the mothers, the results showed that the high rates of exposure persisted between the first and third trimester and that there were not any seasonality effects.

Dr. Schulze stated future work includes a prospective study of aflatoxin exposure and birth outcomes among women in Nepal and a study of aflatoxin exposure and gut integrity in 24 month old children in Bangladesh whose mothers participated in the Iron-Folate versus multiple micronutrient trial.

Dr. Groopman noted that there is an enormous veterinary literature on aflatoxin which could provide insight and direction for work on aflatoxin in humans. His focus has been in China which has regions of high hepatocellular carcinoma (HCC) in which the age of onset and death from HCC is at 45-50, compared with early diagnosis in the U.S. at age 60; Dr. Groopman stated that this early age of onset and death speaks to early life exposures to causative agents. In this regard, aflatoxin is also an early life exposure.

Dr. Groopman stated that of the strategies for prevention of HCC, primary strategies include immunization with the HBV vaccine and reduced aflatoxin consumption, through improvements in food storage and biocontrol, e.g., changes in dietary staples. Secondary strategies include chemopreventive interventions, e.g., oltipraz, broccoli sprouts, chlorophyllin, and green tea.

Dr. Groopman spoke about some of his early chemoprevention strategies which focused on the Nrf2 signaling pathway. Oltipraz, sulforaphane and triterpenoids are chemoprotective inducers of this pathway. This pathway affects a spectrum of target genes that lead to cell survival. These target gene functions include: electrophile detoxication, free radical metabolism, glutathione homeostatis, generation of reducing equivalents, solute transport, proteasome function and inhibition of inflammation. There is tremendous range of potency of the inducers of the Nrf2 signaling pathway; one of the most potent naturally occurring compounds is sulforaphane, which is produced during the digestion (chewing) of broccoli sprouts and can be delivered as a broccoli sprout extract. There are 7 clinical trials in eastern China using chemopreventative approaches involving oltipraz tablets, chlorophyllin tablets and broccoli sprouts tea. Among the findings are that oltipraz increased aflatoxin-mercapturic acid excretion in urine; chlorophyllin decreased aflatoxin-DNA adduct excretion in urine; sulforaphane decreased aflatoxin-DNA adduct excretion in urine; and that broccoli sprout extract modulates metabolism. Dr. Groopman stated that in regions at high risk for HCC, broccoli extract tea can be developed as an intervention for pennies a day by growing the vegetable hydroponically.

Dr. Groopman concluded by noting that 2011 was the fiftieth anniversary of the discovery of aflatoxin. There are about 25 years of molecular toxicology and of validation of well characterized gene products. Aflatoxin is a powerful modifier of epigenic processes and so its effects can go on for generations. Little is known about its early effects.

Discussion

With respect to doing studies in areas where there is a significant HIV prevalence (e.g., in Zimbabwe the HIV prevalence is 15% and transmission is via heterosexual sex), it was noted that most of these persons will be taking anti-retroviral drugs. It was stated that hepatitis C virus (HCV) may also be important as there are certain HCV hot spots in Africa. There are few data on the interaction of HIV and aflatoxin and almost no data on HCV and aflatoxin interaction. It was stated that in Guinea and Ghana, HCV prevalence is very high, but that the issue of interaction with aflatoxin has not been studied. In most other areas of Africa, the prevalence is 2.8%. Dr. Jolly stated that she has some data from prior studies on HCV and aflatoxin and may have data on liver function.

It was stated that in humans, chronic HBV could be the inflammation factor that is driving the pathology. In terms of data on the interaction of aflatoxin and other chronic liver injury agents, such as alcohol, the way the data are usually collected doesn’t allow for assessing this. In a rhesus monkey study, the presence of both
aflatoxin and alcohol did exacerbate liver tumors. It was noted that the area of China in which the broccoli prevention studies are being done, alcohol is primarily used for ceremonial purposes and so may not be a factor in HCC.

In terms of the palatability of broccoli tea, it was noted that at one of the MAL-ED sites, one of first foods children get is a local tea. Dr. Groopman stated that there is a tingling sensation from the sulforaphane in the tea. Lime and pineapple juice are used to sweeten the tea in Asia. In a study in Baltimore, mango juice was used as a sweetener, and no participants dropped out over a 4 months period based on taste issues. He also noted that when the broccoli is boiled, the myrosinase enzyme in the vegetable is killed and the oral and gut enzymes are involved. Diakon from Japanese radish can also provide enzyme. There are variations in the enzymes among individuals. Trying to convert the tea-based strategy to a pill-based strategy could delay an intervention by 4 years because of regulatory issues.

In terms of the sustainability of the broccoli tea strategy, Dr. Groopman noted that since his group has worked in the province, broccoli has become a crop, and importantly, it is a good cash crop. He is looking at community-relevant interventions through the diet. He also stated that it can be more difficult for prevention trials than treatment trials to obtain IRB approval, as in former case the population is healthy and there is concern about adversely affecting healthy people, whereas treatment trials are designed to improve the health of people who are ill.

In response to a question as to whether there might be a downside to the induction of the Nrf2 pathway by the broccoli tea, it was stated that the trial has gone on for 4 months without any adverse effects. Because there is a very high safety bar for prevention trials, the recruitment criteria are very strict and people with poor liver function or any kind of compromised health status are rejected.

It was noted that interventions need to be culturally appropriate and that an intervention that has worked in one region might not be transferrable to other regions. It was noted that are other ways that foodstuffs become contaminated with aflatoxin than in the field; for example, the way that peppers are processed leads to high levels of aflatoxin. In this case, the outer skins of chili peppers have very high levels of aflatoxin.

In order to develop effective interventions, there is a need to understand the mechanisms underlying the effects of mycotoxin. In looking at mechanisms, it is also important to take into account how existing liver and intestinal infections may affect the activation of aflatoxin; for example, acute liver infection may lead to higher activation of aflatoxin. It was stated that fumonisins are promoters of aflatoxin induction of tumors in fish and rat models.

In terms of the source of aflatoxin in Nepal and Bangladesh, it was noted that there is a lot of groundnut consumption in south Nepal and groundnut and maize consumption in Bangladesh.

There was discussion about how to determine the attributable risk for stunting. One suggested method was trying to recapitulate stunting in a model. Insight may also be gained from samples from completed studies which had cancer endpoints and for which there are still materials available and the outcomes of the participants are known. Another approach is do interventions in a case-control approach. Data from case-control studies have shown a linkage of high exposure and growth faltering. However, it was noted that in Brazil, the recent improvement in growth appears to be unrelated in aflatoxin.

In terms of the need for iron supplementation in studies, it was stated that the amount of iron in water varies by region and so each region needed to be assessed as to needs. In some regions of Bangladesh, iron is in water, in other parts of Bangladesh, iron is not in the water and so supplementation is the standard-of-care.

There was discussion of the best way to measure ferritin levels. It was suggested that the alpha-one acid protein says elevated longer than CRP and so is better indicator. Consideration should be given to using
different cut offs for children versus adults. Although measuring ferritin-receptors may help, overall it is difficult determine iron status in the context of an intervention.

**Fumonisin disruption of sphingolipid metabolism: a contributing factor to stunting, malnutrition and enteric diseases in children**  
*Ron Riley*

Dr. Riley indicated that fumonisins are mycotoxins produced by *Fusarium verticillioides* and are common contaminant of corn worldwide. They exert their effects through mechanisms involving disruption of sphingolipid metabolism. He stated that his presentation would cover four areas: (1) fumonisin disruption of sphingolipid metabolism and animal disease; (2) fumonisin in Guatemala; (3) evidence for fumonisin inhibition of ceramide synthase in humans and (4) linking fumonisin disruption of sphingolipid metabolism to human disease.

In order to understand the biological effects that fumonisins could have, it is important to understand sphingolipid biosynthesis and functions. Among the first steps in the biosynthesis pathway is the conversion of palmitoyl CoA + serine into sphinganine, which is then converted to ceramide by ceramide synthase; ceramide is then converted to complex sphingolipids. Fumonisin B1 is a potent inhibitor of ceramide synthases and through the inhibition of this enzyme alters sphingolipid metabolism. This alteration can have three effects. (1) It can lead to altered ceramide signaling pathways and in that way lead to altered cell proliferation and increased apoptosis; (2) It can result in altered functions modulated by complex sphingolipides and in that way resulted in altered lipid raft functions (among their functions, these lipid rafts act to carry folate) as well as altered cell:cell interactions (may bacteria toxins bind to cells via sphingolipids) and cell:matrix interactions. (3) Altered sphingolipid metabolism can lead to altered sphingoid base 1-phosphate signaling pathways affecting sphingosine 1-phosphate (S1P) receptor functions and increased disease risk. Sphinganine 1-phosphate, is usually present in low amounts because it is an intermediate. Sphinganine 1-phosphate can interact with the S1P receptors (there are now five known S1P receptors) and cause disease by affecting a number of pathways including those involved with angiogenesis and vascular maturation; epithelial/endothelial cells permeability; wound healing; altered cell migration and lymphocyte trafficking; and induction of COX-2 in response to inflammatory cytokines. Dr. Riley stated that the goal of this approach is to develop mechanism-based markers, rather than exposure markers, that could be used to link fumonisins to human disease.

Dr. Riley noted that because sphingolipids are important for both the structural and signaling aspects of cells, disruption of sphingolipid metabolism may be the mode of action for fumonisin-induced diseases that affect a broad range of animals, birds, fish and plants. He has also examined the tissues of animals that had all of the diseases that are known to be caused by fumonisins and found that there was elevation of sphingoid base 1-phosphate and sphinganine and accumulation of complex sphingolipids. Dr. Riley stated that fumonisin inhibition also results in the accumulation of deoxysphinganine, which is associated with a human disease, hereditary sensory neuropathy-1, in which there is a genetic defect that results in an enzyme preference for alanine over serine in the interaction of palmitoyl CoA. Persons with this disease make deoxyceramides, which cannot be processed, rather than making ceramides.

Dr. Riley stated that fumonisins are complete carcinogens, so the co-exposure to fumonisins and aflatoxin could have significant biological effects. He stated that the biological importance of the S1P receptors has been recently highlighted by the development of a bacterial toxin-derived drug (fingolimod [FTY720]) for treatment of multiple sclerosis in which the toxin has been modified so that it acts solely on S1P receptors and functions as a potent immunosuppressor.

Dr. Riley stated that there is concern about the relationship of mycotoxins to increased risk of birth defects, including concern about a relationship between maternal ingestion of FB1-contaminated corn during early pregnancy in areas where corn is a dietary staple and increased risk for neural tube defects. He showed
summary data from a mouse model in which pregnant LM/Bc mice were injected with increasing doses of FB1 on gestational days 7.5 and 8.5, and the exposed fetuses were examined for malformations. These data showed that maternal exposure to fumonisins increases the risk for neural tube defects in this animal model and that fumonisins are a contributing factor to neural tube defects, which appears to be multifactorial in origin. Dr. Riley indicated that the CDC is studying neural tube defects in humans in the highlands of Guatemala where the rates of this defect are high.

Dr. Riley stated that if one looks in the red blood cells of these mice, there were large elevations of the sphingoid base 1-phosphate. In these studies, he also evaluated the ratio of sphinganine 1-phosphate to sphingosine 1-phosphate (Sa 1-P/So 1-P) which is what is being used in the human studies.

Dr. Riley summarized work in Guatemala where fumonisin levels in maize sold for human consumption can often be very high. The corn crop is poor; shelling is done by hand, water is in short supply (optimal nixtamalization of corn requires sufficient water), and the corn is poorly stored. Corn is sorted as clean, dirty and rotten. The western central highlands and Pacific lowlands are separated by high mountains with very different altitudes and agroecological environments. There is little fumonisin in the highlands, which are temperate in environment. Fumonisin occurs mostly in the lowlands, which are humid, hot and tropical. Because of the climate, there is insufficient corn production in the highlands for the population and so most of the maize that is consumed in the highlands comes from the lowlands. He noted that like the corn, the people move between the geographic regions. Dr. Riley presented data assembled by Dr. Torres in 2009 relating to stunting in Guatemala; most of the stunting is observed in the western highlands. He noted that the people often move between the highlands and lowlands. In terms of hospital based reporting, Dr. Riley stated that there is likely a great amount of under-reporting as it is difficult for people to get to hospitals.

Dr. Riley showed data on the levels of fumonisin in corn purchased at markets in a number of different departments (administrative regions) in Guatemala in 2005 and 2007. He noted that the levels of fumonisins were the highest in the highlands.

Dr. Riley mentioned the results of a study in Athens that looked at the levels of fumonisins in urine and blood from of people who ate purchased in Athens.

Dr. Riley summarized the unpublished findings that looked at maize-based food consumption by high (> median) and low (< median) consumers; fumonisin levels in urine of high and low consumers; fumonisin levels in the maize and calculated fumonisin B1 intake; urinary fumonisin B1; and the ratio of sphinganine 1-phosphate/sphingosine 1-phosphate (So 1-P/ Sa 1-P) by Guatemalan department. Over 1000 samples were studied and the findings in humans were consistent with the exposures in the various departments. Dr. Riley stated that in terms of excretion, they did not detect any fumonisin FB2 and FB3 in the urine and so it is not clear where these toxins are being excreted. He stated that this is important because JECTA\(^8\) regulates the total amount of fumonisins.

Dr. Riley reported an unpublished regression analysis of urinary fumonisin B1 and the ratio of sphinganine 1-phosphate/sphingosine 1-phosphate for all departments combined and for the departments individually. The data was also assessed by the mean +/- SE urinary fumonisin B1 in each quartile above and below the median ratio of Sa 1-P/So 1-P (0.310) (n=231 to 239/quartile) for all departments combined. It was also assessed by the urinary fumonisin B1 in each quartile above and below the median ratio of Sa 1-P/So 1-P (0.310) as a box plot showing the median, the 25th and 75th and 10th and 90th percentiles and outliers.

Dr. Riley summarized some unpublished data relating to the kinetics of urinary fumonisin B1 excretion in humans consuming maize-based diets. Fumonisin B1 was rapidly absorbed (< 2.75 h to detect in urine); once absorbed, fumonisin B1 levels remained elevated in the urine; the levels rapidly decreased after consumption.

\(^8\) JECFA=Joint FAO/WHO Expert Committee on Food Additives
of foods containing fumonisin B1 ceased ($t_{1/2} > 24 < 72$ h); and fumonisin B$_1$ excretion in the urine was less than 1% of the cumulative dose; and there was a large inter-individual variability.

Dr. Riley posed a number of questions that need to be addressed in future work: Can the sphinganine 1-phosphate/sphingosine 1-phosphate ratio in blood spots and urinary fumonisin be used in epidemiological studies to evaluate the potential of fumonisin as a contributing factor to the high incidence of stunting and diarrheal disease in infants and children? Is co-exposure to fumonisin and aflatoxin common in Guatemala where stunting and/or diarrheal disease in children is high? Is fumonisin acting primarily as an anti-nutritional factor or as a deregulator of S1P receptor functions that regulate basic physiological functions essential for normal growth and development? What are the interventions that could best overcome the anti-nutritional effects and neutralize the adverse effects on cellular regulation?

**Discussion**

Dr. Riley stated that the ratio of sphinganine 1-phosphate to sphingosine 1-phosphate (Sa 1-P/So 1-P) rather than just the S1P level alone, is helpful especially in those cases where the blood spot is not complete as the ratio of these two compounds is constant. This ratio could be developed into a validated biomarker. He noted that S1P alone can be used as is also statistically significant.

In terms of his methodology, Dr. Riley stated that after the specimens are collected in Guatemala, there is some local processing in terms of placing the blood on paper or running the urine through solid phase cartridge; once the specimens are in that form, they are stable for 6 months to a year, respectively. The remainder of the work is done in the U.S. His method is somewhat similar to the one being used by Dr. Gong.

Dr. Riley stated that if a person with a high ratio in their blood moved to an area with low levels of fumonisin, the ratio would drop. In terms of kinetics, the information that is available is from studies in mice using FTY720, which acts as analog of sphingoid base 1-P. In these studies, the mice receive doses of FTY720 and the kinetics are studied. Dr. Rile stated that the compound is gone after 72 hours.

A study in monkeys suggests that FB2 is retained longer than FB1. In a toxicology study in mice, there was no liver damage from FB2 or FB3, but there was an effect from FB1. Findings in rats with naturally contaminated materials also confirm the mouse studies. FB2 and FB3 were not found in the liver or kidney of the rats. The potential mechanism(s) are that FB2 and FB3 are not absorbed or excreted very well.

In terms of fumonisin inhibition of enzymes, most of the inhibition is due to the sphingoid base recognition site.

Dr. Riley stated that for every disease that is driven by fumonisin, there is a need to get above a certain threshold level of exposure before one starts to see disease signs.

Dr. Riley stated that the neural tube in a mouse closes between days 7.5-9.5 of gestation and the animals are dosed during that interval. The mice are examined at day 16 for the presence of NTD.

Given that the SP1 receptor is involved in angiogenesis, the question was raised as to whether fumonisin exposure affects the placenta. It was stated that fumonisin inhibits folate transport (which is associated with lipid rafts). In 1990, at the Texas-Mexico border there was a spike of neural tube defects that was investigated by health authorities. This was at a time in the U.S. when fumonisin levels were extremely high in the Midwest and down through Texas. There was also lot of animal diseases (horses) also being caused by fumonisins at that time. Dr. Riley noted that this confluence of events was how the association of fumonisins and neural tube defects was recognized. Dr. Riley stated that in looking for animal models, he found a specific mouse strain where neural tube defects can be induced at low concentration levels of fumonisins that
would be relevant in terms of oral exposure. He stated that the placenta of the LM/Bc mice is very vacuolated does not look like that of resistant mice. It is possible that the lack of correct functioning of the placenta may be part of the induction of neural tube defects.

In terms of the effect of folate supplementation, if the mice are given folate intraperitoneally and co-exposed to fumonisin, there is 50% reduction in NTP. If ganglioside GM1 is given intraperitoneally, there is almost a 100% protection. It is difficult to do feeding studies using corn culture, but when they are done, the folate doesn’t seem to be very protective. Part of the problem may be that fungus is producing folate and it is hard to analyze the folate in the culture.

It was stated that about 50% of births in Guatemala take place in hospitals and there are a lot of neural tube defects in children. However, there are not fumonisin exposure data available for children in this area.

In other animal model systems with fumonisins, liver and kidney damage is reversible. In horses with neurological damage, once there are neurological symptoms, the process is not reversible. In animals with hyperthorax, the disease is not reversible. There is no reversibility in the mouse NTD model.

Dr. Riley stated that his colleague, Dr. Olga Torres at the Centro de Investigaciones en Nutricion y Salud, Guatemala City, Guatemala has a grant that will look at aflatoxin in corn and maize in terms of stunting in the regions of concern. There isn’t sufficient infrastructure and funding for him to study fumonisin and stunting, and so his work has focused on how fumonisin is contributing to neural tube defects in people who eat large quantities of maize and whose diets are deficient in folate. Depending on the answer and its reproducibility, interventions may be developed.

In terms of aflatoxins outcompeting fumonisins when both are present, Dr. Riley felt that this may not be an issue as fumonisins are pre-harvest contaminants and aflatoxins are post-harvest contaminants. Although he is not doing studies of co-exposure to fumonisins and aflatoxins, it is one of the topics that he listed as questions that need to be answered.

**Deoxynivalenol and the Type B Trichothecene Mycotoxins as Mediators of Growth Suppression and Gut Immunotoxicity**  
*James Pestka*

Dr. Pestka stated that Fusarium and the Type B Trichothecene mycotoxins are sesquiterpenoid metabolites that are produced by Fusarium graminearum in cereal staples worldwide (maize, wheat, barley, sorghum, rice). Deoxynivalenol (DON or “vomitoxin”) is most common of these mycotoxins which bind to ribosomes, inhibit translation and activate stress signaling and are associated with toxicity in animals and humans.

DON and other trichothecenes have been associated with outbreaks of gastroenteritis in humans in many geographic areas, including the “mystery burrito disease” in the US in 1990’s. They target GI function with both acute (anorexia, diarrhea, nausea, vomiting) and chronic effects (growth suppression, decreased weight gain, weight loss). DON has been shown to impair food intake and body weight gain in mice.

Although the greatest concern about DON and other trichothecenes is in food for infants, there are important differences in the way that the U.S. Food and Drug Administration (FDA) and European Union (EU) regulate them. The FDA has a guidance limit of 1000 µg/ kg grain of processed products. There is no tolerable daily intake (TDI) defined and no published risk assessment. By contrast, the EU specifies a maximum limit of 200 - 1250 µg/ kg grain for processed to unprocessed products. The TDI = NOEL\(^9\) x Uncertainty Factor; TDI = 100 µg/kg bw x (1/10) x (1/10) = 1 µg/kg bw. The risk assessment is based on

\[^9\] NOEL = no observed effect level
Concerns have been expressed that Fusarium graminearum could have disastrous effects on cereal crops in the future. These concerns relate to global climate change; the practice of no-till farming to decrease soil erosion, which results in providing fungal inoculums for the next year; the use of high-yield crops, which are more susceptible; the use of non-optimal crop rotation, which also provides more opportunity for fungal infection; and sub-optimal used of fungicides.

Dr. Pestka stated that the MAL-ED project includes several countries in which Fusarium occurs, including India and Brazil.

Dr. Pestka described animal studies related to stunting of children. He hypothesized that the suppression of weight gain and growth is mediated in two ways: (1) by aberrant proinflammatory cytokine upregulation which leads to IGF downregulation and anorexia and (2) by aberrant gut satiety hormone secretion and anorexia.

Dr. Pestka elaborated on these mechanisms at the molecular level. He stated that the innate immune system seems to be very sensitive to DON. DON could act on mononuclear macrophages to cause an increase in cytokines. Also within the mononuclear macrophages, ribosomes act as stress sensors/scaffolds. When DON binds to the ribosomes, it can cause damage/conformational changes which lead to selective gene transcription, increases in mRNA stability, selective mRNA translation and an altered proteome.

Dr. Pestka presented data showing that DON induces proinflammatory gene expression (IL-6, TNF-α, IL-1β) in the spleens, lungs and liver of weanling and adult mice. He also showed data that TLR priming with LPS potentiates DON-induced proinflammatory cytokine expression in mice.

Dr. Pestka provided two possible mechanisms by which DON could act through the brain to suppress weight gain and growth. In the first mechanism, cytokines induced by DON acting on mononuclear phagocytes could act on the brain to induce anorexia, which could lead to decreased weight. Additionally, through the induction of cytokines and SOCS, DON could also act to suppress IGF-1 and the insulin-like growth factor acid-labile subunit (IGFALS) which would also result in decreased weight gain and growth. Dr. Pestka presented data showing the effects of DON on cell signaling and IGF expression. He stated that when DON induces IL-6, it drives growth suppression. From studies in the literature using knockout mice, it is known that IGF-1 and IGFALS deficiencies impair growth. Humans with deficiencies in IGFALS have short stature. The steps in a putative model of DON-induced growth suppression involve inhibition of GH-induced GHR phosphorylation, resulting in reduced IGFALS mRNA transcription, which leads to reduced plasma IGF-1, and then growth suppression.

A second mechanism for suppression of weight gain and growth by DON could also involve effects on gut enteroendocrine cells by increasing the release of satiety hormones. These hormones would then reach the brain and result in anorexia. The gut satiety peptides include Peptide YY (PYY) and Cholecystokinin (CCK). They are produced by enteroendocrine cells in the gut, decreased by fasting and increase after food ingestion. Their effects include the inhibition food intake, reduction of gut motility, and weight loss. Because of these properties, these satiety hormones are being studied for treating obesity. Dr. Pestka showed data demonstrating that DON induces plasma CCK and PYY and anorexia in mice.

Dr. Pestka stated that another toxic effect of DON and the Type B trichothecene mycotoxins that is of special relevance to children is their impairment of the mucosal immune response. Dr. Pestka described studies with Respiratory Enteric Orphan Virus (a double-stranded RNA virus [reovirus]) as a model for mucosal immunotoxicity. This virus has been used as a model system for viral pathogenesis and infects human and animal GI and respiratory tracts. The mucosal immune response to infection is well-characterized. The infection usually occurs early in life, and is self-limited, with GI tract clearance within 7 to 14 days. There is
an innate immune response, cell-mediated immune responses and cytokine production in gastrointestinal-associated lymphoid tissues (GALT) along with induction of virus-specific intestinal IgA and serum IgG. Dr. Pestka presented data showing that DON exposure increases reovirus infection and dose-dependently affects viral copy number in feces and Peyer’s patches.

Dr. Pestka cited two other effects of DON of particular relevance to children: altered gut integrity and nutrient uptake, and impaired in utero development. Dr. Pestka showed data demonstrating that DON consumption impairs development in utero in a rat model where there was increased runting and a decrease in average fetal body weight. He also showed data from rats indicating that DON can cross the placenta.

Dr. Pestka noted that Dr. Paul Turner has been using metabolites of DON to develop DON biomarkers; he provided examples of data using these biomarkers to study DON intake versus urinary DON. Using the DON biomarker, the data show that most people in Europe are exposed to DON at various levels.

In terms of future research needs, Dr. Pestka stated that there is not a good understanding of how DON affects humans. The effects might have a unified pathway with cytokines affecting the liver and brain, and through that the IFG axis, and appetite, growth and weight. Pigs and rodents are possible animal models.

Discussion

Studies of a large archive of samples of cases and controls in Zimbabwe surprisingly showed that IGF1 was much lower in the stunted babies. It was suggested that it is possible that levels in African children are different than in European children.

It was noted that in Latin America, stunted children are still leaving food behind on their plates. There are better ways now to measure food intake and to account for spilled and missed food.

In terms of how the levels of DON intake in humans relate to the FDA guidance and EU regulations, and how the animal data relate to the levels in humans, it was stated that the doses in animals are higher than those in humans, but the range of exposures is wider than the mean that the regulations are based on. In the regulated countries in the EU, about 5% of people are exceeding the recommended levels. In parts of China, the levels in humans are likely to be higher.

Screening for DON started in 1990. During droughts, there had been an expectation of seeing aflatoxin, but that did not occur. DON has been found in the US in grains and in a national brand of granola. DON is now being screened for in grain elevators. In the US, one of big problems is DON in wheat; but recently there has been a problem in corn.

There are almost no data from humans about mycotoxins other than fumonisins, aflatoxin and DON, although these three represent only a minority of the known mycotoxins.

It was also stated that plants can make detoxified forms of DON that protect the plants from DON and its metabolites, but that can affect humans.

In terms of whether hulling removes the DON found in sorghum and rice, it was stated that dehulling can reduce the amounts by 40-50% and that DON is more concentrated in the bran fraction. High fiber bread has more bran and therefore more DON. Heating does not affect DON.

It was stated that toxicity levels are based on animal testing and that is DON metabolized differently by different species of animals, perhaps because of different in gut microbiota. Humans don’t have major detoxification metabolism and so they may be more sensitive to DON.
It was noted that governmental regulation of a factor encourages the development of assays.

It was stated that there are also genetic polymorphisms that may also affect growth; in dogs there is a single polymorphism that affects growth.

**Plenary Session 2: Setting the Research Agenda**

**Key recommendations from Session I- Interventions**

Dr. Jef Leroy provided an overview of the discussions and recommendations related to interventions. He stated that stunting is a major problem affecting over 170 million children, the causation of 65% of stunting remains unknown. World-wide billions of people are exposed to mycotoxin contaminated foods, with the poor being the most vulnerable.

A number of studies have consistently found an association between exposure to aflatoxin through food and the reduction of growth in children. Although the effect sizes are large, the current study designs do not allow for the inference of causality of aflatoxin exposure and stunting because of a number of important confounding factors, such as, age of the children, dietary intake, socio-economic factors and maternal height. These factors are inter-related, e.g. socio-economic status affects the access to food, water, sanitation and health services, these in turn also affect health. Socioeconomic status may also affect the way that crops are managed and food is sorted and stored, which affects the level of aflatoxin exposure, food and nutrient intake and health, all of which determine the overall level of childhood nutrition and stunting. Aflatoxin exposure as a public health issue could be eliminated by changes at the farming and food processing level.

Dr. Leroy suggested that to avoid confounding and get at causality, several approaches could be taken. These include experimental studies, such as randomized control trials (RCT); pseudo-experimental approaches, such as, instrumental variables, (propensity score) matching; developing an in-depth understanding of the biology, pathways, and mechanisms by which mycotoxins act; and by triangulation between some of these methods.

Measurement of exposure to mycotoxins is challenging, and depending on the specific mycotoxin, contamination can occur at one or several stages of food processing, e.g., in the field, at the posho mill, or during home grain storage. Moreover, there is great heterogeneity in levels of contamination and the individual mycotoxins differ in the nature of their effects. For aflatoxin, there are biomarkers for long term exposure in humans and exposure to this mycotoxin is cumulative. However, for fumonisins, there are only biomarkers for short term exposure.

Intervention studies can be useful in a number of ways. They will allow for understanding the association between mycotoxin exposure and child growth, that is, whether the association is causal, and will define the effect size, which reflects how important a particular mycotoxin is as a determinant of stunting. Intervention studies will also inform the design of programs that will lower exposure globally and should include well conducted cost studies. Among the potential outcomes that intervention studies would assess are height-for-age (stunting), weight-for-height (wasting), morbidity, vaccination response, and childhood cognitive development. It is also important to measure the body burden of mycotoxins (e.g., pre- and post-intervention, with and without intervention). The nature of the outcomes might also depend on the target audience.

Dr. Leroy stated that the time and nature of potential interventions depends on the mycotoxin and the crop. For aflatoxin, both pre- and post-harvest interventions may be possible. For fumonisins only pre-harvest interventions are needed.
Pre-harvest approaches include: (1) Employing biocontrol methods, such as use of Aflasafe, that is a mixture of mixture of 4 native atoxigenic strains, in Nigeria; and of Trichodema, which is being used in India; and an emphasis on quality control rather than on making the product accessible to the poorest individuals. (2) Use of resistant cultivars, which have been shown to be effective for groundnuts and for which work is ongoing in maize, and which can be achieved through either traditional breeding or transgenic approaches. (3) Production of “healthy plants” through processes such as, crop management, improved soil fertility, proper soil pH, and soil water retention (through gypsum, lime, compost, crop residue). The key for any intervention is using an integrated approach and recognizing that any solution should lead to higher yields so that farmers have an incentive to adopt the intervention.

Peri- and post-harvest approaches include proper drying that is sufficiently long and off the ground; sorting, which for maize is effective for fumonisin, but is less certain for aflatoxin, and which works quite well for groundnuts including the availability of machinery; use of a rapid screening test; and use of proper storage. Approaches related to consumption include the use of clay (calcium montmorillonite clay [dioctohedral smectite]), which is known to be effective in adults; studies of clay are to be conducted in 3 to 9 year olds; however, this strategy has not been tested in 6 to 36 month old children. Other consumption strategy is the use of chemopreventatives such as green tea polyphenols, chlorophyllin, etc. An area that was not discussed by the participants is the use of demand-side interventions (certification) as a way to incentivize the use of peri- and post-harvest strategies to reduce mycotoxin contamination of products and make them more salable at higher prices.

Other issue discussed by the group was ensuring acceptable mycotoxin levels of processed foods including peanuts that are used to produce products such as RUTF that are used to treat malnutrition in very young children.

**Discussion**

Some participants felt that longitudinal studies can be designed to control for co-variates and that some of the variables mentioned can be measured and controlled for and in that way, causality of mycotoxin exposure and stunting can be addressed.

It was noted that a number of studies have shown that interventions can reduce aflatoxin levels. An important issue is having follow-up to verify that the intervention has been sustained. In this regard, it was noted that follow-up studies of farmers in Malawi which involved a training program with resistant varieties of groundnuts and better post-harvest sorting methods resulted in a product of sufficient quality that allowed for exportation to the UK and sales through a fair-trade organization. However, even in this program, there were some aspects that the farmers could not repeat; for example, they did not have a system to maintain the seed mixture.

In the context of maintaining an intervention, it was stated than an education package was important as was an emphasis on the beneficial effects for children. Empowering the national system right from the beginning of an intervention program through education, awareness, and persistence is another way to ensure the sustainability of an effort. Another factor for sustaining a program relates to the demand side so that the farmer sees a direct benefit through increased consumer prices. In addition to sustainability, another important aspect of an interventional is affordability. Some technologies may not be affordable. However, all interventions, even ones just involving education have a cost and can be expensive. In this regard, it was noted that although vaccines are produced by private companies, they are available to even the very poorest people. Vaccines have the advantage in that they are used a finite number of times for any one person, whereas a chemoprevention strategy might involve a pill a day for a lifetime. In this regard, a change of a harvest or storage practice may be simpler.
It was also noted that an intervention needs to be related to the specific organism and that interventions for long term health benefits are different from interventions designed to investigate causality. A strategy that might be used for investigating causality may involve comparing the amount of stunting seen in populations exposed to toxigenic and atoxigenic strains of fungi.

It was suggested that the availability of a simple publically accessible technology for the consumer to assess the quality of the product might incentivize the production of foodstuffs with less mycotoxin contamination.

It was noted that it was important to consider how an infant’s diet is measured, e.g. breast feeding represents both a way to supply calories, but also represents eating less mycotoxin-contaminated foods as well as providing important immune benefits.

**Key Recommendations from Session 2- Mechanisms and Evidence**

Dr. Felicia Wu provided an overview of the presentations related to mechanisms and evidence for the role of mycotoxins in stunting.

Dr. Ahmed presented data showing that the hub of stunting and underweight is not Africa as is most often though; it is South Asia, where the rate of stunting is 43.2% and that of low birth-weight is 30-40%.

Dr. Ahmed stated that even if the combined interventions of Vitamin A and Zinc, breastfeeding promotion, complementary feeding were adopted at a 99% level, stunting would be reduced by about 33%. It is not clear what other factors account for the rest of the stunting and how much of a role aflatoxin might play in the stunting process. His studies had found that the lactulose:mannitol ratio was not different in stunted versus “normal” children, but this counter-intuitive observation may be related to the cross-sectional study design, the fact that the events of the “normal” child’s life preceding the study were not known, and given the situation in which all of the children were living, the designation of “normal” might not fit any of these children.

Dr. Oswald has presented piglet models involving several mycotoxins and studies of the intestinal barrier, in vivo, ex vivo and at the cellular level. She presented data to show that DON decreases intestinal cell proliferation and decreases weight gain in treated piglets. Cellular studies showed that DON decreases TEER. This effect was dose-dependent and affected the intestinal expression of claudin proteins through MAPKinase activation. Studies also showed that DON induces a Th17-modulated intestinal inflammatory response. Dr. Oswald presented data showing that FB1 impairs the antibody response to vaccines, impacts TEER, and causes intestinal lesions. Aflatoxin leads to the upregulation of IL-6, decreases antigen presenting cell capacity and decreases systemic immune response, thus leading to reduced vaccine response.

Dr. Rudnick addressed pediatric hepatology and the mechanisms of liver recovery. He noted that many mycotoxins are hepatotoxic and that chronic liver injury is associated with growth failure. He proposed the hypothesis that liver injury could mediate biological consequences of mycotoxin exposure, especially growth failure. Dr. Rudnick cited data from clinical experiences and studies from animal models that suggest three mechanisms whereby liver injury could impact growth. These are: inadequate caloric intake and malabsorption, systemic metabolic consequences of chronic liver injury, and the disruption of hepatic growth factor production (IGF-1).

Dr. Turner reiterated that stunting is not fully explained by insufficient diet and infection. He noted that in West Africa there is a prevalence of monotonous diets and a large range of aflatoxin exposure. For example, in The Gambia there is considerable in utero exposure with 100% of women being exposed and aflatoxin being found in 50% cord blood samples. He stated that among the possible mechanisms of aflatoxin action are: liver injury, Zinc bioavailability, maternal toxicity, placental toxicity, and intestinal toxicity. He noted that aflatoxin exposures increase dramatically upon weaning. There is a dose-dependent impact of aflatoxin
on HAZ and WAZ. A simple intervention package (sorting of peanuts, drying them in bags that allowed for air circulation, elevation of the bags and use of insecticides in the storage area floor) reduces AF-alb levels when used in Guinea.

Dr. Schulze provided an overview of nutritional studies in Nepal and Bangladesh. She had found that nutrition was compromised at different levels: Vitamin A, E, Zn, Fe, folate, B12, B6, riboflavin, etc. She had used sera from this population to track AF-lysine adducts in the first and third trimesters of women. She plans to study aflatoxin and birth outcomes in Nepalese women and aflatoxin gastrointestinal impacts in Bangladesh in mothers given an Iron plus Folate supplement versus women given a multiple micronutrient supplement.

Dr. Groopman stated that there has often been a lack of communication between nutritionists and toxicologists. He summarized findings in Jiangsu, China which had shown the association between exposure in early life to aflatoxin and early death from hepatocellular carcinoma (HCC). He noted primary prevention strategies for this would involve pre-harvest and/or post-harvest aflatoxin reduction. Secondary prevention strategies include chemoprevention (e.g. with ITC, triterpenoids, dithiolethiones) and for which trials of broccoli sprouts tea are ongoing in Qidong, China. He noted that the focus of aflatoxin research for the past 50 years has been on cancer because of funding streams; however, that focus has shifted somewhat on other effects of aflatoxin. Given the prevalence of hepatitis B virus (HBV) infection in areas where aflatoxin is in the food, HBV may also have an impact on AF-related stunting. A difficulty in the implementation of chemoprevention trials is the added safety stringency imposed by the IRB criteria for prevention versus therapeutic trials.

Dr. Riley described studies of fumonisin (FB) in Guatemala which showed that this mycotoxin is a cancer promoter and a contributing factor in neural tube defects (NTDs). Mechanistically, FB disrupts sphingolipid metabolism, which has a cascade effect on a number of signaling pathways. Dr. Riley presented data suggesting that FB exposure is linked with stunting, malnutrition, and enteric disease. With respect to NTD development, there are multiple risk factors, including nutritional, genetic, and environmental risks. Dr. Riley also presented data suggesting that the Sa-1-P:S0-1-P ratio can be biomarker of FB exposure; this marker can be measured in blood spots. Regression analysis of urinary levels of FB1 and imputed dietary fumonisin showed a good correlation. Dr. Riley noted that for every disease that is associated with FB, there is a threshold for seeing the effects of the mycotoxin. It also appears that there are possible effects of FB on the placenta and on the occurrence of pre-eclampsia.

Dr. Pestka summarized studies with DON (vomitoxin) and Type B trichothecenes. These mycotoxins are potential problems in several MAL-ED sites. Dr. Pestka summarized work suggesting that DON affects immunity through effects on phagocytes which result in the production of proinflammatory cytokines which further impact the brain and liver resulting in reduced IGF-1 and consequently reduced growth. DON also affects bitter taste receptors, thus affecting the satiety hormones PYY and CKK which affect the brain leading to anorexia and weight loss.

**Discussion**

The participants noted that although the original mycotoxin mechanistic focus on the meeting was on gut/intestinal enteropathy, there is a need to consider affects on the liver as well. (This is done in cancer research where the entire GI tract is considered.) It was also noted that inflammation appeared to be a mechanistic effect that was common across the various mycotoxins. If inflammation is key in terms its effect(s) on growth, consideration should be given to ways to reduce it.

It was noted that chronic inflammation from enteric diseases is a factor that is difficult to separate out in humans from inflammation from other causes. There are also data suggesting that aflatoxin exposure opens the route for infectious agents and so there may be a synergist effect of the combined exposures.
With respect to furthering the interactions between nutritionists and toxicologists, it was suggested that biochemistry can be a common focus. It was noted that nutritionists have studied normal biochemistry in the whole organism, but that the toxicologists have moved apart from them by becoming very focused on subcellular molecular aspects.

It was also suggested that in nutritional studies, there is a tendency to look only at food; however, it is clear that in utero exposures are also important determinants of a child’s development and health.

It was noted that there may be different thresholds for effects of mycotoxins at different key moments in the human developmental process (e.g. for the fetus versus for an infant). There is a lack of information about the relationship of the level of biomarker to the intake dose and lack of information on the relationship of the biomarker levels to functional impact. An example of this is that some children in the highest quartile of exposure to aflatoxin grew to normal size.

With respect to the effect of cumulative doses, it was noted that this is based on studies in rats. In looking at the effect of cumulative doses, consideration should be given to the fact that there is a lot of intrinsic repair that may impact the results.

With respect to standardization of doses and comparison of human and animal doses, it was noted that there are data from pigs and rodents in which maternal levels and their impact on progeny were studied. However, it is uncertain how doses used in animal models relate to doses of mycotoxins to which humans are exposed.

Aflatoxin adducts are important indicators in children, but not adults. In this regard, a single dose of aflatoxin given to young mice results in tumors, perhaps because it is occurring in animals in which there are changes in liver metabolism. It was noted that risk assessment of exposure to BFA in plastics is also related to these early enzyme changes.

It was suggested that the exposure to aflatoxin be looked at from a toxicological perspective; the area under the curve (AUC) for aflatoxin and other toxins may be in the range of 10mg of exposure. This level is an achievable target; that is, if one can reduce the AUC, one could affect the biological impact of exposure. From this perspective, aflatoxin exposure would become a manageable problem. While the dose response is not currently known, it was suggested that from the available biomarker data, it might be possible to back extrapolate and obtain this number. In this regard, there are a lot of data from the studies of aflatoxin and cancer studies and it is likely that the number would not be off by more than 100 grams.

In nutritional studies in developing countries, it is important to consider how much of a diet is from foods that can contain mycotoxins; in this regard, animal source foods do not contain toxins and also have the property of promoting growth.

The observation that stunted children may still leave food on the table could be related to some of the anorexic effects that have been described by the meeting participants.

With respect to the usefulness of vaccination of children with HBV vaccine, it was stated that the vaccine itself has a positive effect and is worthwhile independent from any HBV infection interactions with mycotoxins. It was stated that there is some preliminary data suggesting that early HBV infection modulates aflatoxin metabolism and from this perspective may increase the impact of a particular level of aflatoxin exposure.

Stunting in children results from a complex web of events and causes and is therefore a difficult outcome measure. When doing randomized trials for mycotoxin exposure, the best that one may be able to do is to ‘take the mycotoxin away,” which will also cause other ripple effects. The need to be able to compare across
studies was also noted. Some investigators have a list of factors that have to be assessed. Factors that have not been mentioned a lot during the meeting include the effects of water and hygiene. It would be important to assess additional domains in the critical 1000 first days of life, such as, the prevalence of infections (ID rates), dietary diversity (animal sources versus vegetable sources), feeding of breast milk and the exclusivity of breast feeding, sanitation and hygiene conditions, sufficiency of micronutrients and macronutrients, and mothers status (e.g., height, weight, BMI). In this regard it was noted that many of the infections that are present are not manifested as problems in terms of symptomatology. The participants noted that the cause of stunting is clearly multifactorial and that one needs to look at the problem as a whole and develop more broad-based solutions.

All mycotoxins disrupt signaling pathways. Although each mycotoxin may have specific primary pathway targets, these pathways overlap and intersect. If one looks at the proximal effect/target for some mycotoxins, e.g. adducts, when looking for stunting as an outcome, the factors that intervene could be quite different and could be related to contributing factors (particular agent, oxidative stress). However, it is important to recognize that secondary effects may also determine outcomes.

In the MAL-ED project, 54 different microorganisms as well as diet are being studied; aflatoxin was not in original study. The amount of aflatoxin-related work that can be done is limited by the amount of blood that is collected and available.

In terms of encouraging breastfeeding for 1000 days for areas where breastfeeding is often that long, maternal education was noted as an important factor. It was also noted as a factor that is important for many of the aspects that the group has been discussing.

It was noted that while aflatoxin biomarkers have been correlated to intake, the exact relationship of the biomarker to intake is not clear. A possible simple plan is to replace the current food of a small group of children with clean food. This might be achieved also by the use of preventive strategies such as broccoli tea or clay.

It was noted that in West Africa, an important factor related to stunting is how soon the subsequent sibling is born; many children are being born because so many children are dying. If women had fewer children and could breast feed them for 3 years, a large part of the stunting problem might be avoided.

Although the focus has been on three mycotoxins, there are many more in nature and so it is not clear which ones are most important and should be studied. It was suggested that it is important to first assess the mycotoxins that are in the food of the population that one plans to study. There are 6 to 9 prominent mycotoxins. There is a laboratory in Austria that can measure 58 components at one time.

It was noted that environmental, dietary, biological and many other factors are in play in affecting peoples exposures to compounds that are deleterious to their health. In Africa, people exposed to PAH from both food and smoking. For example, people heat their food on hot asphalt, and smoke maize and peanuts for insect control.

Other Issues Raised by Meeting Participants

Although there was not a specific session related to this, during the course of the presentations and discussion periods, the participants identified a number of gaps, obstacles, opportunities, and recommendations toward developing a better understanding of the impact of mycotoxins on gut function and stunting. These issues have been summarized below.

Gaps
Only 35% of stunting of children can be attributed to known factors. While there is solid association of stunting with exposure to mycotoxins, the causality has not been proven and the percentage of stunting attributable to mycotoxins in general or to specific mycotoxins is not known.

Although there is a body of information on aflatoxins, fumonisins and DON, it is known that there are many more mycotoxins to which humans are exposed and for which there is little information. Mechanisms for the effects of aflatoxin, fumonisins and DON on humans have been put forth, but there is much less information about the effects of DON on humans. There is a need to understand the mechanisms of action of mycotoxins in order to develop effective interventions.

There is little knowledge about the potential synergy between/among mycotoxins in those geographic areas in which food is contaminated with multiple mycotoxins.

There is a need to characterize the effect of mycotoxins on the intestinal microbiota and a need to understand how mycotoxins interact with acute and chronic infections that are endemic in the population.

There is a need for better understanding of the effects of mycotoxins on the human immune system. There seems to be a commonality in mycotoxin action in terms of causing inflammation, although the molecular pathways may be different for specific mycotoxins. In examining the effects of mycotoxins on the immune response, it may be important to look at T cell subsets so as to detect differential effects.

There is a need for clear definition of the critical time points in the perinatal period for the adverse effects of aflatoxin exposure on child growth in West Africa. No studies have measured exposure through pregnancy and into the first few years of life. No studies have measured intestinal damage, aflatoxin, and growth in cohorts.

In terms of understanding the biological effects of fumonisins, further work is needed to determine if the sphinganine 1-phosphate/sphingosine 1-phosphate ratio can be used in epidemiological studies and to determine whether fumonisin is acting primarily as an anti-nutritional factor or as a deregulator of S1P receptor functions.

There is a need for firm estimates of the DALYs and economic burden of mycotoxin exposure so as to raise more awareness in international agencies and governmental bodies about the impact of mycotoxins on health and the need for resources to address this problem.

There is insufficient knowledge on how social networks or education/radio campaigns can affect the knowledge about aflatoxin and how they might be useful in turning knowledge and awareness to appropriate actions to reduce the prevalence levels of aflatoxin.

**Obstacles**

Stunting is likely to reflect a multifactorial process (nutrition, environment, genetics, infections, maternal factors) and thus teasing out the aspects related to any specific factor is difficult.

Exposure to mycotoxins is a complex measure, as there may be effects from both acute toxic exposures as well as cumulative effects, especially for aflatoxin which is fat soluble. The dosage that leads to biological effects in humans may differ because of the multifactorial nature of some of the effects.

Existing infections of the liver and gut can affect the activity of mycotoxins.
Although there are several methodologies for measuring aflatoxin in body fluids, the measures use different instrumentation and detect different subsets of related molecules, which makes absolute comparisons across studies difficult. Never the less, the directionality and relative effect sizes do have a consistency across studies.

The biomarkers for DON and fumonisin have only recently been developed. For Fumonisin biomonitoring data remain limited to a couple of studies in high risk settings, while for DON the biomarker has been used more frequently including high and low risk settings in China, in addition to the UK, Egypt, France and Sweden. However no biomarker driven epidemiology has been conducted to date.

It was noted that the environmental enteropathy results both in changes in permeability and in reduced surface area in the intestines. Although there are measures for the changes in permeability, the reduced surface area can also be an additional factor and it is not clear if there is a way to measure this.

The levels of mycotoxin exposure and the effect sizes observed vary in different geographic regions and national settings. Additionally, these exposures occur in the context of different nutritional and micronutrient deficits as well as on a background of acute and chronic infections.

Funding for mycotoxin-associated stunting has been difficult to obtain through the National Institutes of Health; in the past mycotoxins have more often been studied in the context of carcinogenesis than in the context of stunting.

Although the EU has defined regulatory levels of exposure, the FDA has provided only guidance in this area.

Historically, there has been a lack of communication between nutritionists and toxicologists, which in the past has impacted on study designs and lack of sufficient concurrent consideration of nutritional and toxicological factors in developing investigations and interventions.

**Opportunities**

Mycotoxin exposure is known to be a problem in areas in which there are MAL-ED study sites, thus there is the potential for using the infrastructure and studies of the MAL-ED project or companion studies to provide insights into the effects of mycotoxin exposure.

The Meridian Institute has just completed a grant agreement with the BMGF to support the Africa-led, Partnership for Aflatoxin Control in Africa (PACA). The grant includes support for a number of activities including country assessments, scaling up of beneficial fungi (Aflasafe), improvement and implementation of low-cost methods for post-harvest storage, drying and handling, and studies of economic impact and health. Companion studies might be built on this project.

There are ongoing clinical trials in Malawi and Ghana supported by the BMGF that are enrolling women during pregnancy. Although these trials are not designed to address mycotoxins, there may be an opportunity for companion studies with these ongoing trials.

Specimens are available from a variety of studies conducted for other purposes (nutritional studies, cancer epidemiology, cancer prevention) in areas of the world where mycotoxin-associated stunting is known to occur. There has been long-term follow-up of some of the participants in these studies and there is the potential to investigate levels of aflatoxin in these specimens and to correlate them with health-related outcomes.
Exposure to aflatoxin could be looked at from a toxicological perspective; the area under the curve (AUC) for aflatoxin and other toxins may be in the range of 10mg of exposure. This level is an achievable target; that is, if one can reduce the AUC, one could affect the biological impact of exposure. From this perspective, aflatoxin exposure would become a manageable problem. While the dose response is not currently known, it was suggested that from the available biomarker data, it might be possible to back extrapolate and obtain this number. In this regard, there are a lot of data from the studies of aflatoxin and cancer studies that could be used.

**Recommendations**

In considering the mechanisms by which mycotoxins may cause stunting, the liver should be included rather than just an exclusive focus on the gut.

It is also important to take into account how existing liver and intestinal infections may affect the activation of aflatoxin; for example, acute liver infection may lead to higher activation of aflatoxin.

In discussing the effects of mycotoxins, it is important to distinguish between toxicology and exposure. In studying aflatoxin, it is important to determine if the strains are S or L as there is a difference in toxicity of these two types.

Although each mycotoxin may have specific primary pathway targets, these pathways overlap and intersect. It is therefore important to consider not only the proximal effect/target, but also important to recognize that secondary effects may also determine outcomes to exposure.

In looking at causality of stunting it is important to recognize its multifactorial nature and to look at the problem as a whole and develop more broad-based solutions.

An important issue that needs to be addressed is whether intervention approaches to restrict aflatoxin exposure can alleviate mycotoxin exposure of infants and restrict growth faltering. This kind of intervention study may provide strong causality data and promote more global intervention strategies.

Intervention studies will inform the design of programs that will lower exposure globally and should include well conducted cost studies.

In intervention studies, it is important to measure the body burden of mycotoxins (e.g., pre- and post-intervention, with and without intervention).

In designing studies related to stunting in children, investigators need to focus both on their “must study” factors, but also consider measuring factors that will allow for comparison across studies and that recognize the multifactorial nature of stunting. It would be important to assess important domains in the critical 1000 first days of life, such as, the prevalence of infections (ID rates), dietary diversity (animal sources versus vegetable sources), feeding of breast milk and the exclusivity of breast feeding, sanitation and hygiene conditions, sufficiency of micronutrients and macronutrients, and mothers status (e.g., height, weight, BMI).

Randomized controlled trials are needed to determine the effectiveness of education and awareness campaigns in changing behavior with respect to adopting risk mitigating practices.

It is important for trials to have complementing work done on market access.
Summary

The meeting participants emphasized the enormous global burden of disease attributable to maternal and child under-nutrition. In this context, the adverse sequelae of mycotoxin exposure through contaminated food is particularly high in those parts of the developing world where the population’s diet is limited to mainly a single food stable that is vulnerable to fungal infection, with the major period of vulnerability being prenatally and in the first few years of life. Consumption of aflatoxin-contaminated food is associated with lifelong impediments to health and development, including cognitive impairments, susceptibility to disease, and reduced response to vaccinations. Although direct causality and the mechanisms by which mycotoxins cause these outcomes are not fully understood and they are likely to involve multi-system, multi-organ as well as organ-specific effects, there is accumulating evidence for the role of inflammation and the adverse impact on intestinal integrity as important factors. Interventions face a number of barriers including the need to be tailored to the specific agroecological, economic, regulatory, educational, and social milieu of the country of deployment as well as to complex ethical and safety issues related to use of preventative strategies. Never the less, some simple promising strategies are currently under study. Further work is needed in the area of mechanisms so that they can be taken into account in designing epidemiological studies and intervention strategies. Although biomarkers and assays are available for aflatoxin, more work in this area is needed for other mycotoxins as well as work to compare results across assays for specific mycotoxins.

While animal models are available, the one most similar to humans, the piglet, is limited in terms of the number of animals that can be studied. While there are a number of rodent models, these are limited by the differences in the metabolic and immune systems of rodents from those of humans. Studies to assess the burden, mechanisms, and intervention strategies are labor intensive and need an in-country infrastructure that requires time and resources to develop. Thus, the potential for using extant samples from prior studies or building add-on studies to ongoing projects, such as MAL-ED, will be important for efficiently obtaining the knowledge needed to understand and intervene in the adverse health outcomes associated with mycotoxin exposure.

Appendices:

Agenda

List of Participants
# AFLATOXIN: Impact on Stunting in Children and Interventions to Reduce Exposure

**February 1-2, 2012**

**Conference Room 4ABC, IFPRI, Washington, DC**

| DAY 1  
Wednesday, February 1 | ACTIVITY | FACILITATOR/PRESENTER |
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<tbody>
<tr>
<td><strong>7:45 – 8:15</strong></td>
<td><strong>BREAKFAST</strong></td>
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| **8:15 – 8:30**        | **Introduction**  
Welcoming participants | Maximo Torero and Gretchen Meller |
| **8:30 – 8:40**        | Overview of Stunting: Determinants and Unknown Contributors | Marie Ruel |
| **8:40 – 9:00**        | Estimates of Global Exposure | Felicia Wu |
| **9:00 – 9:20**        | Highlights from Aflaccontrol Project: Aflatoxin Prevalence Levels and Household Knowledge, Attitudes, and Perceptions | Clare Narrod |
| **9:20 – 9:40**        | Risk Factors for Aflatoxin Exposure and Child Stunting: Identifying Key Covariate Contributors in Two Studies | Kitty Cardwell |
| **9:40 – 10:10**       | Discussion | Jennifer Pratt Miles |
| **10:10 – 10:30**      | **COFFEE/TEA BREAK** |                       |
| **10:30 – 11:15**      | Understanding the Connection between Malnutrition and Enteric Disease: Overview of the Mal-Ed Project Objectives and Data Sets | Dennis Lang |
| **Field studies: 20 minute presentations, 40 minutes discussion** |                       |                       |
| **11:15 – 12:15**      | Role and Mechanisms of Aflatoxin in Maternal Anemia, Low Birth Weight and Stunting Among Infants and Children in Africa | Pauline Jolly |
| **12:15 – 1:00**       | **LUNCH** |                       |
| **1:00 – 2:00**        | Mycotoxin Exposure, Impact on Child Health and Intervention in African Children | Yun Yun Gong |
| **Concurrent Session, Group A:**  
Interventions to Reduce Aflatoxin Exposure – Measuring Exposure and Establishing Casualty  
(30 minute presentations; 20 minute discussion)  
**Conference Room 6A** |                       |                       |
| **2:00 – 2:50**        | Posho-Mill Survey of Aflatoxin and Fumonisins Levels in Kenyan Maize | Rebecca Nelson |
| **2:50 – 3:10**        | **COFFEE/TEA BREAK** |                       |
| **3:10 – 4:00**        | Mycotoxin Contamination of Maize is Associated with Stunting and Underweight in Children Aged 12-59 Months in Kenya | Laura Smith |
| **4:00 – 5:00**        | Establishing Causality between Aflatoxin Exposure and Stunting  
Wrap-up of the day | Jef Leroy and Kelly Jones |
<table>
<thead>
<tr>
<th>Time</th>
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<th>Presenter</th>
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<tbody>
<tr>
<td>2:00 – 3:00</td>
<td>Is Childhood Stunting in Bangladesh Due to Exposure to Aflatoxin?</td>
<td>Tahmeed Ahmed</td>
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<td>3:00 – 3:15</td>
<td><strong>COFFEE/TEA BREAK</strong></td>
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<tr>
<td>3:15 – 4:15</td>
<td>The Effect of Different Mycotoxins on the Intestinal Barrier Function and on the Immune Response</td>
<td>Isabelle Oswald</td>
</tr>
<tr>
<td>4:15 – 5:00</td>
<td>Mycotoxin Liver Injury in Children: Regenerative Hepatobiology as a Model for Human Growth Failure from Mycotoxin Exposure</td>
<td>David Rudnick</td>
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**Dinner**

6:00  | **KELLARI TAVERNA: 1700 K St. NW**
     | (4 blocks away between N 17th St & N Connecticut Ave)                |
## AFLATOXIN: Impact on Stunting in Children and Interventions to Reduce Exposure

**February 1-2, 2012**  
**Conference Room 4ABC, IFPRI, Washington, DC**

| DAY 2  
Thursday, February 2 | ACTIVITY                                                                 | FACILITATOR/ PRESENTOR |
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<td><strong>Morning</strong></td>
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<td>7:30 – 8:00</td>
<td><strong>BREAKFAST</strong></td>
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| Concurrent Session, Group A:  
Interventions to Reduce Aflatoxin Exposure (30 minute presentations; 20 minute discussion)  
Executive Room       | 8:00 – 8:50 Clay-Amended Nutritional Supplements to Mitigate Aflatoxicosis and Diarrhea in Infants and Children in Ghana | Timothy D. Phillips    |
|                      | 8:50 – 9:40 Comprehensive Assessment of Maize Aflatoxin Levels in Eastern Kenya, 2005–2007 | Lauren Lewis           |
| 9:40 – 10:00         | **COFFEE/TEA BREAK**                                                      |                        |
|                      | 10:00 – 10:50 Aflatoxin Mitigation in Africa                              | Ranajit Bandyopadhyay  |
|                      | 10:50 – 11:40 Reducing Aflatoxin Exposure Through On-Farm Management Practices | Farid Waliyar          |
| Concurrent Session, Group B:  
Understanding the Impact of Mycotoxins on Gut Function and Stunting: Mechanisms & Biomarkers (20 minute presentations: 40 minutes discussion)  
Conference Room 4ABC | 8:00 – 9:00 Aflatoxin and Growth Faltering: Potential Mechanisms | Paul Turner            |
|                      | 9:00 – 10:00 Human Toxicology of Aflatoxin and Potential Impacts in Micronutrient Deficient Populations | John Groopman and Kerry Schulze |
| 10:00 – 10:20        | **COFFEE/TEA BREAK**                                                      |                        |
|                      | 10:20 – 11:20 Fumonisin Disruption of Sphingolipid Metabolism: A Contributing Factor to Stunting, Malnutrition and Enteric Diseases in Children | Ron Riley              |
|                      | 11:20 – 12:20 Deoxynivalenol and the Type B Trichothecene Mycotoxins as Mediators of Growth Suppression and Gut Immunotoxicity | James J. Pestka        |
| 12:20 – 1:20         | **LUNCH**                                                                 |                        |
| **Afternoon**        |                                                                           |                        |
| 1:20 – 1:50          | Plenary Session 2: Setting the Research Agenda                             |                        |
|                      | Present Key Recommendations from Session 1 (Interventions)                 | Jef Leroy              |
|                      | 1:50 – 2:20 Present Key Recommendations from Session 2 (Mechanisms and Evidence) | Felicia Wu             |
|                      | 2:20 – 3:00 Discussion                                                    | Jennifer Pratt Miles   |
|                      | 3:00 – 4:00 Collaborative Work Session to Draft LOIs (Optional)            |                        |
### Aflatoxin and Stunting Meeting
**Washington, DC on February 1-2, 2012**
#### List of Participants

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